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National Bureau of Standards
Center for Analytical Chemistry
Organic Analytical Research Division
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In studies to evaluate the potential health and ecological effects of atmospheric emissions, bioassays have been employed in conjunction with chemical characterization to correlate mutagenic and/or carcinogenic activity with chemical composition. The complexity of an air particulate extract necessitates the prefractionation of the mixture into suitable subfractions or chemical classes prior to chemical characterization and/or biological testing. The goal of this project was to evaluate such a fractionation scheme for air particulate material with respect to chemical characterization of the various fractions with particular emphasis on the identification of polycyclic aromatic hydrocarbons (PAH). In this study we have used three chromatographic approaches to separate, identify, and quantitate the complex mixture of PAH extracted from SRM 1649 (Urban Dust/ Organics): (1) capillary gas chromatography (GC), (2) liquid chromatography (LC) with selective fluorescence detection, and (3) multidimensional chromatographic techniques. The analytical methods used for the certification of several PAH in SRM 1649 are described in this report. In addition, over 80 PAH, including many alkyl-substituted PAH, are tentatively identified using GC, LC, and gas chromatography-mass spectrometry (GC-MS) data. Preliminary results of biological testing on selected fractions isolated from the urban dust sample are reported.

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SECTION 1

INTRODUCTION

In studies to evaluate the potential health and ecological effects of atmospheric emissions (e.g., air particulate matter), bioassays such as the Ames test have been employed in conjunction with chemical characterization to correlate mutagenic and/or carcinogenic activity with chemical composition. The complexity of an air particulate extract necessitates prefractionation of the mixture into suitable subfractions or chemical classes prior to chemical characterization and/or biological testing. Classically, air particulate extracts have been divided into acidic, basic, and neutral fractions; the neutral fraction is then generally further divided into non-polar neutral, aromatic, and polar neutral subgroups. The goal of this project was to evaluate such a fractionation scheme for air particulate material with respect to the chemical characterization of the various fractions with particular emphasis on the determination of polycyclic aromatic hydrocarbons.

Polycyclic aromatic compounds (PAC) are widespread environmental pollutants produced by incomplete combustion and pyrolysis of fossil fuels and other organic materials. A recent book by Lee *et al.* (1) and a review by Bartle *et al.* (2) provide excellent discussions concerning the determination of these compounds. During the past thirty years, many studies have been undertaken to characterize the polycyclic aromatic hydrocarbon (PAH) content of airborne particulate matter (2, and references 36-68 therein). Previous studies have focused on the PAH constituents mainly owing to the known carcinogenic activity of many of these compounds. Other subgroups of PAC reported in air particulate samples include polycyclic aromatic sulfur heterocycles (PASH), polycyclic aromatic nitrogen heterocycles (PANH) also known as aza-arenes, nitropolycyclic aromatic hydrocarbons (NPAH), polycyclic aromatic oxygen heterocycles (PAOH), and polycyclic aromatic quinones (PAQ). [These acronyms are assigned according to the format defined by Bartle *et al.* (2)]. In this work we have focused on methods for the determination of PAH, PASH, and PANH in the air particulate material.

Compound structure, position of substitution, and presence and position of a heteroatom in the ring have all been found to affect the carcinogenic and/or mutagenic properties of these aromatic compounds (1), and therefore, the exact structural elucidation of individual components of a mixture is necessary to determine which compounds are responsible for the carcinogenic hazard of the sample. PAC mixtures from air particulate samples are extremely complex because of the presence of alkyl-substituted PAC as well as the numerous isomeric parent compounds. As a result, the determination of PAC components in such mixtures requires the use of high efficiency

chromatographic separation techniques and selective detection systems for accurate identification and quantitation.

Urban air particulate material collected in Washington, D.C. (see Experimental section) was used for the evaluation of the various fractionation schemes. Using analytical methods developed at NBS for the determination of PAH, this urban particulate sample has been certified for the concentrations of selected PAH and has been issued recently as Standard Reference Material (SRM) 1649 "Urban Dust/Organics". The analytical methods used for certification of this SRM are described in this report.

AIR PARTICULATE FRACTIONATION SCHEMES

A number of organic solvents including acetone, benzene, toluene, methylene chloride, methanol, and cyclohexane have been recommended for the extraction of air particulate samples (3-7). Acetone, benzene, and cyclohexane have all been reported to be nearly 100% efficient in the extraction of benzo[a]pyrene (3). Cyclohexane has often been employed for extraction of air particulate matter for the determination of PAH. However, more polar solvents such as benzene and methanol are required to remove the more polar organic constituents. Recently, Grimmer et al. (7) compared the extraction efficiency of several solvents for removing PAH from air particulate filters and found toluene to be the most effective. Dong et al. (6) used benzene: methanol (4:1) to extract air filters in the determination of aza-arenes. Since aza-arenes are frequently weak bases, salt formation with inorganic and organic acids is a possibility which necessitates the use of the more polar solvent (6).

The extraction of air particulate matter with an organic solvent results in a complex mixture of organic and inorganic constituents. This mixture must be fractionated into subgroups prior to chemical characterization. A number of fractionation schemes have been used for air particulate extracts, primarily for the isolation of PAH components (1-2, 8-10). These procedures involved chromatographic isolation on silica and/or lipophilic gels and liquid-liquid partition with nitromethane.

A general fractionation scheme for the characterization of the organic constituents extracted from air particulate material is illustrated in Figure 1. In general, most fractionation schemes designed for characterization of all groups of organic compounds involve partition with dilute alkali and acid to separate, respectively, acidic (e.g., phenols) and basic components (aza-arenes), from the neutral components as shown in Figure 1. The neutral fraction is further separated into nonpolar neutrals (aliphatics), aromatic (PAH and PASH), and polar neutral fractions by silica column chromatography or liquid-liquid partition.

CHARACTERIZATION OF POLYCYCLIC AROMATIC HYDROCARBONS (PAH) FROM AIR PARTICULATE MATTER

In recent years a number of papers have reported the determination of PAH in air particulate samples (1,2). The majority of these papers have reported only qualitative information on the PAH content of particulate samples; however, several studies illustrate the use of gas chromatography (GC) (7,11-13) and liquid chromatography (LC) (13-15) for the quantitation of selected PAH extracted from air particulate matter.

Solvent extraction of air particulate matter provides a complex mixture of organic constituents from which the PAH must be isolated prior to identification and quantitation. The PAH mixtures isolated from air particulate extracts are extremely complex because of the presence of numerous alkylated PAH as well as the numerous isomeric parent PAH. The complexity of these PAH mixtures necessitates the use of high resolution gas and/or liquid chromatographic techniques with selective detection [e.g., fluorescence or mass spectrometry (MS)] to achieve separation, identification, and quantitation of individual components. Recent reviews on the use of capillary gas chromatography (16) and high performance liquid chromatography (17) for the determination of PAH have appeared. We have used three chromatographic approaches to separate, identify, and quantitate the complex PAH mixture isolated from the Washington dust sample: [1] high resolution capillary GC with flame ionization detection (FID); [2] LC with selective fluorescence detection; [3] multidimensional chromatographic techniques (i.e., normal-phase LC to isolate specific PAH fractions followed by GC, GC-MS, and/or reversed-phase LC). The first and second approaches were used for quantitation of selected major PAH components in the extract after appropriate sample cleanup. These first two approaches were used for the certification of Standard Reference Material (SRM) 1649 "Urban Dust/Organics". This material is issued in 10-g quantities with certified and information values for the concentrations of selected PAH. SRM 1649 is a companion material to SRM 1648 "Urban Particulate Matter" which is available in 2-g quantities and certified for inorganic constituents. The third approach was used primarily for a detailed characterization of both the major and minor PAH components. The third approach requires isolation and analysis of various fractions and thus is more time-consuming than the first two approaches.

SECTION 2

EXPERIMENTAL

SAMPLE PREPARATION AND ANALYSES FOR CERTIFICATION OF SRM 1649

The air particulate sample was collected in the Washington, D.C. area using baghouses specially designed for this purpose. This material was collected over a period in excess of 12 months and, therefore, represents a time-integrated sample. While this sample is not intended to be representative of air particulate samples from the area in which it was collected, it should typify the analytical problems associated with the chemical analysis of atmospheric particulate matter collected in an urban area. The particulate material was removed from the filter bags by a specially designed vacuum cleaner and combined into a single lot. This material was subsequently screened through a fine mesh sieve (120 mesh) to remove bag fibers and other extraneous material. The sieved material was then thoroughly mixed in a V-blender, bottled, and the bottles sequentially numbered. Randomly selected bottles were used for the analytical measurements. Sample aliquots of 1 g were extracted in a Soxhlet extractor for 48 h with a cycle time of about 20 min. Samples prepared for GC analysis were extracted with 450 mL of a 1:1 mixture of benzene/methanol, whereas samples for LC analysis were extracted with a similar volume of methylene chloride. An internal standard solution of 1-methylpyrene (for GC analysis) or 7-methylfluoranthene and/or perylene-d_{12} (for LC analysis) was added to the particulate samples prior to extraction. The perylene-d_{12} ($\geq$98%) was obtained from MSD Isotopes (Merck & Co., Rahway, NJ); 7-methylfluoranthene was obtained from the National Cancer Institute Chemical Carcinogen Repository (IIT Research Institute, Chicago, IL); 1-methylpyrene was obtained from ICN K&K Laboratories (Plainview, NY). The internal standard solutions were prepared in methylene chloride and 1 mL of the solution was added onto the dust samples in the Soxhlet extraction thimble. Prior to GC analysis the extract was concentrated in a rotary evaporator, redissolved in cyclohexane, and partitioned between N,N-dimethylformamide (DMF) and water as described by Bjørseth (12). After the liquid-liquid partition the total PAH fraction was isolated by normal-phase LC on an aminosilane column (30 cm x 9 mm i.d., μBondapak NH_2, Waters Associates, Milford, MA) using 3% methylene chloride in hexane as the mobile phase. The PAH fraction was collected, concentrated, solvent changed to toluene, and analyzed by GC (Model 3700 Gas Chromatograph, Varian Associates, Palo Alto, CA) on a fused silica column (30 m x 0.25 mm i.d.) coated with a 0.25 μm film thickness of SE-52. The sample (1 μL sample plus 1 μL toluene solvent flush) was injected using an on-column injector (J & W Scientific, Rancho Cordova, CA). The carrier gas for the GC analyses was hydrogen. Flame ionization detection was used in all GC analyses.

Sample clean-up for the LC analyses consisted of concentration, solvent exchange to cyclohexane, followed by a liquid-liquid partition between cyclohexane and nitromethane (4). The nitromethane solution was concentrated to approximately 1 mL and diluted with 1 mL tetrahydrofuran. The LC analyses [Varian Model 5000 Liquid Chromatograph with Vista CDS 401 (Varian Associates, Palo Alto, CA) and a Model 440 UV absorbance detector at 254 nm (Waters Assoc., Milford, MA)] were performed on a 5 μm C_{18} column (Vydac 201TP, The Separations Group, Hesperia, CA) with a solvent gradient from 40% acetonitrile in water to 100% acetonitrile in 45 min at 1.5 mL/min. A variable wavelength fluorescence detector (Model 3000, Perkin Elmer Corp., Norwalk, CT) was used which is capable of changing excitation and emission wavelength conditions during the chromatographic run (three sets of excitation and emission wavelength conditions are stored). Detector response factors for the quantitation of the selected PAH were obtained by analysis of SRM 1647 — Priority Pollutant Polynuclear Aromatic Hydrocarbons in Acetonitrile (18) — in conjunction with the air particulate extract. The standard compounds which were not included in SRM 1647 (i.e., triphenylene (≥97%), perylene (≥99%) and benzo[e]pyrene (>99%)] were obtained from Fluka/Tridom Chemical Inc., (Hauppauge, NY) and the Community Bureau of Reference (Brussels, Belgium).

SAMPLE PREPARATION FOR CHARACTERIZATION BY MULTIDIMENSIONAL CHROMATOGRAPHY

The two PAH fractions which were used for normal-phase LC fractionation to obtain samples for subsequent analyses by reversed-phase LC and GC-MS were isolated as described below. The sample (∿15 g) which was used for reversed-phase LC analysis was Soxhlet extracted for 24 h with benzene/methanol (1:1) and fractionated by acid-base partition (see Figure 1) to obtain a neutral fraction which was then partitioned with cyclohexane/DMF/H_2O to obtain an enriched PAH fraction. This fraction was then separated on the amino column to obtain the desired fractions. The sample (∿21 g) which was used for GC-MS analysis was Soxhlet extracted for 96 h with cyclohexane. Cyclohexane was used for extraction of this particular sample to avoid removing significant quantities of more polar constituents. The extract was then partitioned with DMF/H_2O followed by a silica gel cleanup on a short column (SepPak silica cartridge Waters Associates, Milford, MA). The enriched PAH fraction was then separated on the amino column to obtain the desired fractions.

GAS CHROMATOGRAPHY/MASS SPECTROMETRY (GC/MS) ANALYSES

GC/MS analyses were performed on a Hewlett-Packard 5985 system equipped with an HP 1000 computer (Hewlett Packard Co., Palo Alto, CA). Chromatographic separations were carried out on a 30 m x 0.25 mm i.d. fused silica capillary column coated with a 0.25 μm film of SE-52 (J & W Scientific, Inc., Rancho Cordova, CA). The column was interfaced directly to the ion source of the mass spectrometer. Helium was used as the carrier gas. All GC/MS runs were carried out with a 2 μL injection and 1:10 split. The initial oven temperature was 200 °C for 2 min, programmed at 4 °C/min to a final temperature of 275 °C and then held at this temperature for 10 to 60 min depending on the fraction being analyzed. The mass spectrometer was operated in the electron

impact mode with an electron energy of 70 eV and an ion source temperature of 200 °C. Mass spectra were scanned repetitively at 200 amu/s over the entire chromatographic run.

SECTION 3

RESULTS AND DISCUSSION

QUANTITATION OF MAJOR PAH BY GC AND LC

The certification of an SRM requires the use of two independent and reliable analytical methods (19). The results from these two independent methods must agree within a specified uncertainty in order for the values to be certified. In the case where agreement is not within the specified limits or where only one method of quantitation was employed, the values are reported only as informational values. In order to provide two independent analytical methods in the determination of selected PAH in the Washington air particulate material, different sample extraction solvents, internal standards, and cleanup steps were used prior to the chromatographic separation and quantitation. GC and LC were used as the two different chromatographic techniques for separation and quantitation.

GC Analysis

Prior to analysis by GC, the PAH fraction was isolated from the complex mixture using a liquid-liquid partition with cyclohexane/DMF/water followed by a normal-phase LC separation to isolate the total PAH fraction (see discussion to follow). A gas chromatogram of the PAH fraction isolated in this manner from the Washington urban dust (SRM 1649) is shown in Figure 2. The major PAH were quantified using 1-methylpyrene as an internal standard. As shown in Figure 2, the major peaks were the unsubstituted parent PAH. The majority of the smaller peaks are alkyl-substituted PAH as will be discussed later.

LC Analysis

In the LC analysis of the Washington dust extract, fluorescence detection was used to achieve the selectivity necessary to quantify the individual PAH components in the complex mixture without extensive preseparation or cleanup. Because of the selectivity for PAH of LC fluorescence detection compared to the universal FID for GC, a less rigorous PAH isolation procedure was used for the LC analyses than for the GC analyses (i.e., cyclohexane/nitromethane partition vs. cyclohexane/DMF/water partition and normal-phase LC).

The fluorescence spectral characteristics of a number of PAH are summarized in Table 1. By selection of the appropriate excitation and emission wavelengths, a high degree of specificity can be achieved. This spectral selectivity often permits the determination of individual PAH in a mixture

TABLE 1. FLUORESCENCE SPECTRAL CHARACTERISTICS OF PAH[a]

	Fluorescence Excitation Spectra λ, nm								Fluorescence Emission Spectra λ, nm					
Phenanthrene	249	273	280	292					345	353	362	382		
1-Methylphenanthrene	248	253	278	286	298				350		367	388	408	
3,6-Dimethylphenanthrene	247(s)	253	277	286	297				353		370	391	413(s)	
Anthracene	250	308	320	337	355	374			376	398	423	448		
Fluoranthene	260	275	280	284	307(s)	332	340	356	463	(425-475)				
Pyrene	261	274	290(s)	305	318	333			370	377	382	387	390	
1-Methylpyrene	233(s)	242	254(s)	264	275	312	325	340	374			395	415	
Triphenylene	245	254	269	281					344	351	358	367	377	387(s)
Benz[a]anthracene	255	265	275	285	324	337	355		384	406	432	460		
Chrysene	255	263	282	303	318				360	378	401	424		
1-Methylchrysene	260	268	287	298	310	324			362	380	403	427		
4-Methylchrysene		272		302	316	328			368	387	408	432		
Benzo[e]pyrene	266	276	286	302	315	328			374	385	394	406	417	
Benzo[j]fluoranthene	290	312	305	328	362	381			507	(490-520)				
Benzo[b]fluoranthene	255	274	289	296	348				433	(415-460)				
Perylene	251	263(s)	363	383	406				435	463	498			
Benzo[k]fluoranthene	245	266	294	304	357	376			407	430	457(s)			

TABLE 1. (Continued)

	Fluorescence Excitation Spectra λ, nm								Fluorescence Emission Spectra λ, nm				
Benzo[a]pyrene	265	284	297	348	363	383			404	427	453		
Dibenz[a,c]anthracene[b]	269(s)	279	289						377	388	398	409(s)	
Dibenz[a,h]anthracene	286	295	317	332	347				392	402(s)	414	439	470(s)
Benzo[b]chrysene	275	284	302						394	417	445		
Picene[b]	287	304	328						377	398	421	449	
Benzo[ghi]perylene	272(s)	284	295	326	343	357			404	413	417	427	441(s)
									455(s)	460(s)			
Indeno[1,2,3-cd]pyrene	239	247	273	288(s)	298	311	356	372	472	496	(465-515)		
	380	404											
Anthanthrene[b]	260	296	308	384	401	407	422	430	432	459	494		
Dibenzo[g,p]chrysene[b]	280	292	303	340	353				395	409			
Dibenzo[b,def]chrysene[b]	272	310	312	399	422	428			451	480	518		
Naphtho[1,2,3,4-def]chrysene[b]	276	293	305	330	342	358	376		397	408	420	446	
Benzo[rst]pentaphene[b]	247-395	274	285	297	316	332	355	373	434	450	462	480	494

[a]Note: The most intense peak is underlined with solid line, peaks which have intensity greater than 70% of the most intense peak are underlined with broken line. Shoulders are indicated by (s). Broad peaks are followed by wavelength range in which the intensity is greater than approximately 80% of the maximum. Spectra were obtained in 80/20 acetonitrile/water mixture and are uncorrected.

[b]Data from Reference 20, spectra were obtained in cyclohexane.

even when complete liquid chromatographic resolution of the components is not achieved. The selectivity of LC analysis with fluorescence detection is illustrated in Figure 3 for the Washington dust sample. In Figure 3 the upper chromatogram is the reversed-phase LC analysis of a total PAH fraction (obtained from normal-phase LC isolation procedure as described later) with UV detection. UV detection provides a nearly "universal" LC detector for PAH. The major PAH are discernable in this chromatogram; however, accurate quantitation of the majority of these components would be difficult due to incomplete chromatographic resolution. The middle and lower liquid chromatograms in Figure 3 are the LC analyses of the same Washington dust total PAH fraction using fluorescence detection at the wavelength conditions described in Table 2. These chromatograms illustrate the specificity achievable with fluorescence detection optimized for various PAH.

TABLE 2. FLUORESCENCE CONDITIONS FOR LC DETERMINATION OF PAH IN AIR PARTICULATE SAMPLES

	Wavelengths (nm) (see Figure 3)		
	excitation	emission	PAH Quantitated
λ_1	250	360	phenanthrene
λ_2	285	450	fluoranthene, 7-methylfluoranthene (I.S.)[a], benzo[b]fluoranthene
λ_3	295	400	benzo[k]fluoranthene, benzo[a]pyrene, benzo[ghi]perylene
λ_4	335	385	pyrene
λ_5	285	390	benz[a]anthracene, dibenz[a,h]anthracene
λ_6	270	360	chrysene
λ_7	400	440	perylene, benzo[k]fluoranthene

[a] I.S. = internal standard.

In the measurement of selected PAH for the certification of SRM 1649, three LC runs, each with three or four sets of wavelength conditions (one in each run specific for the internal standard) were used for the quantitation of 11 PAH. The reversed-phase liquid chromatograms for these three runs with fluorescence detection and a single run with UV detection are shown in Figure 4. The fluorescence detection conditions are given in Table 3. Even with only minimal sample cleanup (no normal-phase LC as compared to Figure 3), the selectivity of the fluorescence detection allows quantitation of individual PAH.

TABLE 3. FLUORESCENCE CONDITIONS FOR THE LC DETERMINATION OF SELECTED PAH IN WASHINGTON PARTICULATE MATTER

	Wavelengths (nm) (see Figure 4)		
	excitation	emission	PAH Quantitated
λ_1	285	450	fluoranthene and 7-methylfluoranthene (I.S.)[a]
λ_2	400	440	perylene-d_{12} (I.S.) and perylene
λ_3	295	405	benzo[k]fluoranthene, benzo[a]-pyrene, and benzo[ghi]perylene
λ_4	330	385	pyrene
λ_5	285	385	benz[a]anthracene, dibenz[a,h]-anthracene, and benzo[ghi]perylene
λ_6	290	360	phenanthrene
λ_7	270	360	chrysene
λ_8	300	500	indeno[1,2,3-cd]pyrene

[a] I.S. = internal standard.

The results of the GC and LC determinations are summarized in Table 4. The LC results are presented separately for the measurements using 7-methylfluoranthene and perylene-d_{12} as internal standards. It was observed that after a number of analyses, the LC column would lose some resolution between the internal standard peak (7-methylfluoranthene) and a minor peak in the sample. This resolution loss resulted in greater variability in the results. Thus, a different internal standard, perylene-d_{12}, was used for the remaining analyses. In general, the results in Table 4 illustrate the improved analytical precision for the results from LC method II compared to LC method I. Data for some PAH are not reported in Table 4 for several reasons. For example, GC values are not reported for triphenylene, benzo[b]fluoranthene, benzo[k]fluoranthene, and dibenz[a,h]anthracene because of co-elution with other compounds (e.g., chrysene and triphenylene). LC values for triphenylene and benzo[e]pyrene were not reported owing to the lack of sensitivity and/or selectivity for these two compounds using fluorescence detection. Triphenylene was measured in three samples only after a normal-phase isolation of the four condensed ring PAH fraction. Results for phenanthrene and dibenz[a,h]anthracene were reported only for method LC-II which used more selective fluorescence conditions than were used in method LC-I.

TABLE 4. SUMMARY OF RESULTS BY THE VARIOUS ANALYTICAL METHODS FOR THE WASHINGTON URBAN DUST (SRM 1649)

Compound	Concentration (µg/g) GC [4][a]	LC-I [18]	LC-II [9]
Phenanthrene	---	---	4.5 ± 0.3[b] (9)[c]
*Fluoranthene	7.3 ± 0.2[b] (4)	7.0 ± 0.5[b] (24)	6.8 ± 0.4 (9)
Pyrene	7.2 ± 0.2 (4)	6.3 ± 0.4 (17)	6.2 ± 0.2 (9)
Chrysene	4.6 ± 0.2[d] (4)	3.5 ± 0.1 (5)	3.7 ± 0.2 (9)
*Benz[a]anthracene	2.4 ± 0.1 (4)	2.8 ± 0.3 (18)	2.4 ± 0.1 (3)
Triphenylene	---	---	1.7 ± 0.1[e] (3)
Perylene	0.84± 0.09 (4)	0.80± 0.04 (17)	0.65± 0.02 (9)
Benzo[e]pyrene	3.3 ± 0.2 (4)	---	---
*Benzo[a]pyrene	3.0 ± 0.3 (4)	2.6 ± 0.4 (18)	2.6 ± 0.1 (9)
Benzo[b]fluoranthene	---	6.2 ± 0.3 (18)	---
Benzo[k]fluoranthene	---	2.0 ± 0.1 (18)	2.1 ± 0.1 (9)
*Benzo[ghi]perylene	4.7 ± 0.2 (4)	3.9 ± 0.8 (12)	5.2 ± 0.6 (9)
Dibenz[a,h]anthracene	---	---	0.41± 0.07 (9)
*Indeno[1,2,3-cd]pyrene	3.3 ± 0.3 (4)	3.4 ± 0.4 (16)	3.6 ± 0.2 (9)

[a] Number in [ ] indicates number of samples extracted.

[b] Uncertainty is one standard deviation of the mean.

[c] Number in ( ) indicates number of measurements.

[d] GC determination includes triphenylene which coelutes with chrysene.

[e] Triphenylene determined by LC analysis of four ring PAH fraction isolated by normal-phase LC.

* Indicates compounds with certified values in Table 4A.

Note: LC-I used 7-methylfluoranthene as the internal standard and LC-II used perylene-d_{12} as the internal standard.

The certified values for five PAH are given in Table 4A. Certified values are provided only when the results of the two independent methods agree within specified uncertainty. Pyrene and perylene were not certified because of lack of suitable agreement between the methods. The values for the remaining PAH were determined by only one method and were, therefore, not certified but are provided only for information.

TABLE 4A. CERTIFIED VALUES FOR SELECTED POLYCYCLIC AROMATIC HYDROCARBONS IN SRM 1649

Compound	Concentration (μg/g)[a]
Fluoranthene	7.1 ± 0.5
Benz[a]anthracene	2.6 ± 0.3
Benzo[a]pyrene	2.9 ± 0.5
Benzo[ghi]perylene	4.5 ± 1.1
Indeno[1,2,3-cd]pyrene	3.3 ± 0.5

[a]The estimated uncertainty listed for a constituent is the union of 95% confidence intervals computed separately for each analytical method and represents an evaluation of the combined efforts of method imprecision, possible systematic errors among methods, and material homogeneity. The estimated uncertainty is intended to correspond to approximately 95% confidence limits.

SRM 1648, "Urban Particulate Matter", which was collected in the St. Louis area, was also analyzed by LC for the determination of 9 PAH. The results are summarized in Table 5. The concentrations of most of the major PAH were slightly higher in the St. Louis sample compared to the Washington sample. The St. Louis collection site was selected as representative of an industrial area, whereas the Washington site was selected as representative of an area with less industrial input. Qualitatively, the results for the PAH in the St. Louis and Washington sample were very similar indicating that automobile exhaust may be the principle source of PAH in the particulate samples.

TABLE 5. DETERMINATION OF SELECTED PAH IN ST. LOUIS URBAN AIR PARTICULATE MATTER (SRM 1648) (μg/g)[a]

Compound	Concentration (μg/g)[a]
Fluoranthene	7.9 ± 0.6[b]
Pyrene	7.4 ± 0.2
Chrysene	6.6 ± 0.1
Benz[a]anthracene	2.8 ± 0.1
Perylene	0.65 ± 0.02
Benzo[k]fluoranthene	3.3 ± 0.1
Benzo[a]pyrene	2.6 ± 0.2
Benzo[ghi]perylene	5.5 ± 0.8
Indeno[1,2,3-cd]pyrene	4.8 ± 0.2

[a]Three 1-g samples of particulate matter were extracted.

[b]Uncertainties are ±1σ from the mean.

MULTIDIMENSIONAL CHROMATOGRAPHY FOR CHARACTERIZATION OF PAH MIXTURES

For the detailed qualitative characterization of the complex PAH mixture from the Washington particulate samples, a multidimensional chromatographic approach was employed. Multidimensional chromatography is the sequential combination of at least two different chromatographic modes of separation to analyze complex mixtures, e.g., LC/GC, LC/LC, TLC/GC, etc. The major PAH constituents in the air particulate extract were found to be the unsubstituted PAH with smaller amounts (<10 percent of the parent PAH) of the alkyl-substituted PAH. In order to isolate and identify the numerous minor components in the PAH mixture, a normal-phase LC procedure on an aminosilane column was used to separate the PAH according to the number of aromatic carbons (21-22). The normal-phase retention characteristics of a number of PAH and PASH on an aminosilane column are listed in Tables 6 and 7. In normal-phase LC, isomeric PAH have similar retention characteristics e.g., benz[a]anthracene, chrysene, and triphenylene have retention indices of 4.00, 4.01, and 4.07 (A difference of 0.1 retention index units is required for complete resolution of two compounds. See reference 21 for a complete discussion of LC retention indices for PAH). Alkyl-substituted PAH elute in the same region as the parent PAH as shown in Table 6. This normal-phase LC procedure provides PAH fractions which contain isomeric PAH and their alkyl substituted homologs. These fractions were then analyzed by GC-MS and reversed-phase LC. Reversed-phase LC provides excellent selectivity for the separation of isomeric PAH and alkyl substituted PAH (17,22-23) e.g., see the methylchrysene isomers in Table 6. Reversed-phase LC retention characteristics for over 90 PAH have been reported and discussed by Wise *et al*. (22-23).

TABLE 6. LIQUID CHROMATOGRAPHIC RETENTION OF POLYCYCLIC AROMATIC HYDROCARBONS (Logarithim of the Retention Index)[a]

Compound	Mol. wt.	No. of Aromatic C	Normal-Phase[b] NH_2 Column	Reversed-Phase[c] C_{18} Column
Naphthalene	128	10	2.00	2.00
Fluorene	166	12	2.55	2.70
Anthracene	178	14	2.94	3.20
2-Methylanthracene	192	14	3.01	3.71
9-Methylanthracene	192	14	3.02	3.39
9,10-Dimethylanthracene	206	14	3.08	3.63
Phenanthrene	178	14	3.00	3.00
1-Methylphenanthrene	192	14	3.02	3.38
2-Methylphenanthrene	192	14	3.00	3.72
3-Methylphenanthrene	192	14	3.12	3.32
9-Methylphenanthrene	192	14	3.02	3.31
1,8-Dimethylphenanthrene	206	14	3.11	3.79
Benzo[a]fluorene	216	16	3.51	3.72
Benzo[b]fluorene	216	16	3.54	3.84
4H-Cyclopenta[def]phenanthrene	190	14	3.10	3.16
Pyrene	202	16	3.37	3.48
1-Methylpyrene	216	16	3.46	3.90
2,7-Dimethylpyrene	230	16	3.47	4.40
Fluoranthene	202	16	3.51	3.37

Table 6. (continued)

Compound	Mol. wt.	No. of Aromatic C	Normal-Phase[b] NH_2 Column	Reversed-Phase[c] C_{18} Column
Benz[a]anthracene	228	18	4.00	4.00
1-Methylbenz[a]anthracene	242	18	3.90	4.14
5-Methylbenz[a]anthracene	242	18	4.04	4.28
6-Methylbenz[a]anthracene	242	18	4.03	4.10
8-Methylbenz[a]anthracene	242	18	4.03	4.19
9-Methylbenz[a]anthracene	242	18	4.08	4.39
11-Methylbenz[a]anthracene	242	18	3.91	4.13
Chrysene	228	18	4.01	4.10
1-Methylchrysene	242	18	4.07	4.43
2-Methylchrysene	242	18	4.08	4.52
3-Methylchrysene	242	18	4.12	4.29
4-Methylchrysene	242	18	3.95	4.18
5-Methylchrysene	242	18	3.94	4.35
6-Methylchrysene	242	18	4.10	4.14
Triphenylene	228	18	4.07	3.70
Benzo[c]phenanthrene	228	18	3.64	3.64
Benzo[ghi]fluoranthene	226	18	3.84	3.95
Benzo[b]fluoranthene	252	20	4.48	4.29
Benzo[j]fluoranthene	252	20	4.56	4.24
Benzo[k]fluoranthene	252	20	4.45	4.42
Benzo[a]pyrene	252	20	4.38	4.68
Benzo[e]pyrene	252	20	4.46	4.28

Table 6. (continued)

Compound	Mol. wt.	No. of Aromatic C	Normal-Phase[b] NH_2 Column	Reversed-Phase[c] C_{18} Column
Perylene	252	20	4.61	4.33
Benzo[ghi]perylene	276	22	4.83	4.73
Anthanthrene	276	22	4.80	4.93
Indeno[1,2,3-cd]pyrene	276	22	4.90	4.83
Dibenz[a,c]anthracene	278	22	4.93	4.40
Dibenz[a,h]anthracene	278	22	4.94	4.72
Benzo[b]chrysene	278	22	5.00	5.00
Picene	278	22	5.03	5.10

[a]Retention reported as logarithm of the retention index, see reference 21.

[b]n-hexane as the mobile phase.

[c]Mixtures of 70 or 85 percent acetonitrile in water as mobile phase.

TABLE 7. LIQUID CHROMATOGRAPHIC RETENTION OF POLYCYCLIC AROMATIC SULFUR HETEROCYCLES (Logarithm of the Retention Index)[a]

Compound	Mol. wt.	No. of Aromatic C	Normal-Phase[b] NH_2 Column
Dibenzothiophene	184	12	2.87
Benzo[b]naphtho[1,2-d]thiophene	234	16	3.65
Benzo[b]naphtho[2,1-d]thiophene	234	16	3.59
Benzo[b]naphtho[2,3-d]thiophene	234	16	3.78
Chryseno[4,5-bcd]thiophene	258	18	4.04
Benzo[2,3]phenanthro[4,5-bcd]-thiophene	258	18	3.83
Triphenyleno[4,5-bcd]thiophene	258	18	4.09
Dinaphtho[1,2-b:1',2'-d]thiophene	284	20	4.33
Dinaphtho[1,2-b:2',1'-d]thiophene	284	20	4.40

[a]See reference 21-22 for discussion of retention index system.

[b]n-hexane as mobile phase.

The normal-phase separation of the PAH mixture isolated from the Washington dust (see experimental section for details of the isolation) is shown in Figure 5. The reversed-phase liquid chromatograms of these fractions (including a total PAH fraction) are shown in Figure 6 and 7 to illustrate the usefulness of this approach for the analysis of complex mixtures. Using UV absorption detection at 254 nm (generally considered as a "universal" detector for PAH), quantitation of even the major PAH in the total PAH fraction would be difficult owing to the complexity of the mixture. However, the normal-phase LC preseparation based on the number of aromatic carbons provides fractions suitable for LC analysis even with the "universal" UV detector. Fraction 2 contains the three condensed ring isomers, phenanthrene and anthracene, and alkyl substituted phenanthrenes/anthracenes; fraction 3 contains the peri-condensed four ring isomers, fluoranthene and pyrene, and their alkyl substituted isomers. The four ring cata-condensed isomers of triphenylene, benz[a]anthracene, and chrysene are found in fraction 4. Quantitation of triphenylene in the air particulate samples (see Table 4) was achieved by reversed-phase LC analysis of fraction 4. Chrysene and triphenylene were not resolved in the GC analyses. Fraction 5 contains the isomers of molecular weight 252, i.e., benzo[b]fluoranthene, perylene, benzo[k]fluoranthene, and benzo[a]pyrene. The six condensed ring isomers of molecular weight 276, benzo[ghi]perylene and indeno[1,2,3-cd]pyrene, are the major peaks in fraction 6. The PAH components in these fractions were identified based on LC retention times and fluorescence spectra compared with standard compounds.

All of the chromatograms in Figures 6 and 7 were obtained using UV detection. By using fluorescence detection in the analysis of these fractions,

additional selectivity could be achieved. For example, fraction, 3, which contains pyrene/fluoranthene and alkyl substituted isomers, could be analyzed with fluorescence conditions selective for only pyrene and alkylpyrenes (excitation-333 nm, emission-370 nm) or selective for only fluoranthene and alkylfluoranthenes (excitation-284 nm, emission-463 nm).

For detailed characterization of the numerous minor PAH and PASH, normal-phase LC isolation of the various fractions followed by GC and GC-MS analysis of these fractions was used. Lee et al. (24) reported the GC-MS analysis of LC fractions from gel chromatography on LH-20 for the determination of PAH from air particulate material. The normal-phase separation on an aminosilane column of the PAH isolated from the air particulate extract by liquid-liquid partition and silica cleanup (see experimental section for details) is shown in Figure 8. The individual fractions were separated by GC and identified based on GC retention times (compared with standard compounds) and mass spectra. Information from LC analyses of these same fractions was also used for identifications. The gas chromatograms of the individual fractions are shown in Figure 9 to 17 and the peak identifications are listed in Table 8.

Fraction 1 contains fluorene and dibenzothiophene as the major parent PAH and PASH. Methylfluorenes and C_1- through C_4-substituted dibenzothiophenes are the remaining constituents. Both fluorene and dibenzothiophene have 12 aromatic carbons and elute prior to phenanthrene in the normal-phase LC separation (retention indices of 2.55 and 2.87, respectively). Phenanthrene is the major peak in fraction 2. Anthracene is present, but only at about the same level as several of the methylphenanthrenes. 4H-cyclopenta[def]phenanthrene and the sulfur containing analog of molecular weight 208 are also included in fraction 2 as would be expected by the number of aromatic carbons.

The isomeric pyrene and fluoranthene were isolated into separate LC fractions, 2b and 3, (LC retention indices of 3.37 and 3.51, respectively) for the GC analysis whereas they were combined in one fraction for the reversed-phase LC analysis (see Figure 6, fraction 3). Minor components in the pyrene fraction include alkylsubstituted 4H-cyclopenta[def]phenanthrene isomers and methylpyrenes. The fluoranthene fraction (no. 3) contains methylfluoranthenes, benzofluorenes, benzo[ghi]fluoranthene, benzo[c]phenanthrene and three PASH of molecular weight 234, i.e., benzo[b]naphtho[1,2-d]thiophene, benzo[b]-naphtho[2,1-d]thiophene, and benzo[b]naphtho[2,3-d]thiophene. All of the PAH and PASH in this fraction have 16 aromatic carbons except benzo[c]phenanthrene and benzo[ghi]fluoranthene. These two PAH elute earlier than expected based on aromatic carbon number because of their compact structure.

The four ring cata-condensed PAH found in fraction 4 include benz[a]-anthracene, chrysene, triphenylene, C_1- and C_2-substituted molecular weight 228 isomers, methylbenzo[ghi]fluoranthene, and possible PASH of molecular weight 258. The major peaks in fraction 5 are the five ring peri-condensed isomers of molecular weight 252 (i.e., benzo[b]fluoranthene, benzo[k]-fluoranthene, benzo[e]pyrene, benzo[a]pyrene, and perylene), three isomers of molecular weight 276, and a compound of molecular weight 306 (possibly quaterphenyl). Benzo[ghi]perylene and anthanthrene (six ring peri-condensed PAH appear in fraction 5, but could have been fractionated into fraction 6 (as in Figure 6, fraction 6) or a separate fraction based on the retention

TABLE 8. POLYCYCLIC AROMATIC HYDROCARBONS IDENTIFIED IN WASHINGTON URBAN PARTICULATE MATTER[a]

Peak No.	Mol. wt.	Compound
Fraction 1		
1	166	Fluorene[b,c]
2 (a,b)	180	Methylfluorene[b]
3	184	Dibenzothiophene[b,c]
4 (a-c)	198	Methyldibenzothiophene[b]
5 (a-i)	212	C_2-substituted dibenzothiophene[b]
6 (a-g)	226	C_3-substituted dibenzothiophene[b]
7	240	C_4-substituted dibenzothiophene[b]
Fraction 2		
1	184	Dibenzothiophene[b,c]
2	178	Phenanthrene[b,c,d]
3	178	Anthracene[b,c,d]
4	192	3-Methylphenanthrene[b,c]
5	192	2-Methylphenanthrene[b,c]
6	192	2-Methylanthracene[b,c]
7	190	4H-Cyclopenta[def]phenanthrene[b,c]
8	192	1-Methylphenanthrene[b,c]
9 (a-i)	206	C_2-substituted phenanthrene/anthracene[b]
10	208	Phenanthro[4,5-bcd]thiophene[b,c]
11	218	C_2-substituted 4H-cyclopenta[def]phenanthrene[b]
12 (a,b)	218	C_2-substituted 4H-cyclopenta[def]phenanthrene[b]
13 (a,b)	232	C_3-substituted 4H-cyclopenta[def]phenanthrene[b]

TABLE 8. (Continued)

Peak No.	Mol. wt.	Compound
Fraction 2b		
1	204	C_1-substituted 4H-cyclopenta[def]phenanthrene[b]
2 (a-d)	206	C_2-substituted phenanthrene/anthracene[b]
3 (a,b)	218	C_2-substituted 4H-cyclopenta[def]phenanthrene[b]
4	202	Pyrene[b,c,d]
5	204	C_1-substituted 4H-cyclopenta[def]phenanthrene[b]
6	218	C_2-substituted 4H-cyclopenta[def]phenanthrene[b]
7	216	2-Methyl- or 4-methylpyrene[b,c]
8	218	C_2-substituted 4H-cyclopenta[def]phenanthrene[b]
9	216	1-Methylpyrene[b,c]
10	218	C_2-substituted 4H-cyclopenta[def]phenanthrene[b]
11	244	C_3-substituted pyrene/fluoranthene[b]
12	258	unknown
13	242	unknown
Fraction 3		
1	202	Fluoranthene[b,c,d]
2	202	Acephenanthylene[b,e]
3	202	Pyrene (overlap from Fraction 2b)[b,c]
4	230	unknown
5	218	C_2-substituted 4H-cyclopenta[def]phenanthrene[b]
6	230	C_2-substituted fluoranthene/pyrene[b]
7	216	Methylfluoranthene[b,f]
8	216	Methylfluoranthene[b,f]
9	216	Benzo[a]fluorene[b,c]
10	216	Benzo[b]fluorene[b,c]
11	216	Methylfluoranthene[b,f]
12	216	Methylfluoranthene[b,f]
13	216	Methylfluoranthene[b,f]
14 (a-e)	230	C_2-substituted fluoranthene/pyrene[b]
15	234	Benzo[b]naphtho[2,1-d]thiophene[b,c]

TABLE 8. (Continued)

Peak No.	Mole. wt.	Compound
Fraction 3 (Continued)		
16	226	Benzo[ghi]fluoranthene[b,c]
17	228	Benzo[c]phenanthrene[b,c]
18	234	Benzo[b]naphtho[1,2-d]thiophene[b,c]
19	234	Benzo[b]naphtho[2,3-d]thiophene[b,c]
20	228	Benz[a]anthracene (overlap with Fraction 4)[b,c]
	226	Cyclopenta[cd]pyrene[b,c]
21	228	Chrysene (overlap with Fraction 4)[b,c]
Fraction 4		
1	234	Benzo[b]naphtho[1,2-d]thiophene isomer[b]
2	234	Benzo[b]naphtho[1,2-d]thiophene isomer[b]
3	228	Benz[a]anthracene[b,c]
4	228	Chrysene[b,c]
	228	Triphenylene[b,c]
5	242	Methylchrysene/benz[a]anthracene/triphenylene[b]
6	242	Methylchrysene/benz[a]anthracene/triphenylene[b]
7	242	Methylchrysene/benz[a]anthracene/triphenylene[b]
8	240	Methylbenzo[ghi]fluoranthene[b]
9	242	Methylchrysene/benz[a]anthracene/triphenylene[b]
10	256	C_2-substituted 228 isomer[b]
11	268	unknown (methylbinaphthyl)[b]
12	258	unknown
	268	unknown (methylbinaphthyl)[b]
13	268	unknown (methylbinaphthyl)[b]
14	258	unknown
	268	unknown (methylbinaphthyl)[b]

TABLE 8. (Continued)

Peak No.	Mol. wt.	Compound
Fraction 5		
1	242	Methyl-substituted 228 isomer[b]
2	254	Binaphthyl[b]
3	254	Binaphthyl[b]
4	254	Binaphthyl[b]
5	256	C_2-substituted 228 isomer[b]
6	252	Benzofluoranthenes[b,c]
7	252	unknown
8	252	Benzo[e]pyrene[b,c,d]
9	252	Benzo[a]pyrene[b,c,d]
10	252	Perylene[b,c,d]
11	266	Methyl-substituted 252 isomer or dibenzofluorene isomer[b]
12	306	Quaterphenyl[b]
13	266	Methyl-substituted 252 isomer or dibenzofluorene isomer[b]
14	266	Methyl-substituted 252 isomer or dibenzofluorene isomer[b]
15	264	unknown
16 (a,b)	284	C_2-substituted 252 isomer[b]
17	276	unknown
18	276	Benzo[ghi]perylene[b,c,d]
19	276	Anthanthrene[b,c]
Fraction 6		
1	300	unknown
2	276	Indeno[1,2,3-cd]Fluoranthene[b,c]
3	276	unknown
4	276	Indeno[1,2,3-cd]pyrene[b,c,d]
5	276	Benzo[ghi]perylene (overlap from fraction 5)[b,c]

TABLE 8. (Continued)

Peak No.	Mol. wt.	Compound
Fraction 7		
1	278	Dibenz[a,j]anthracene[b,c,d]
2	278	Dibenz[a,c]anthracene and dibenz[a,h]-anthracene[b,c,d]
3	278	unknown
4	278	Benzo[b]chrysene[b,c,d]
5	278	Picene[b,c,d]
6	290	unknown
7	292	Methyl substituted 278 isomer[b]
8	304	unknown
9	302	Dibenzopyrene[b]
10	302	Dibenzopyrene[b]
11	302	Dibenzopyrene[b]
12	300	Unknown
13	300	Unknown
Fraction 8		
1	302	Dibenzopyrene isomer[b]
2	302	Dibenzopyrene isomer[b]
3	302	Dibenzopyrene isomer[b]
4	302	Dibenzopyrene isomer[b]
5	302	Dibenzopyrene isomer[b]
6	302	Dibenzopyrene isomer[b]

[a] Identifications are best estimates based on mass spectral data, GC and LC retention data compared to standard compounds, and fluorescence spectra compared to standard compounds.

[b] Identification based on MS.

[c] Identification based on GC retention.

[d] Identification based on LC retention and fluorescence spectra.

[e] Reference [25].

[f] Identified as methyl fluoranthene rather than methylpyrene based on normal-phase LC retention.

indices of these compounds, i.e., 4.61 for perylene compared to 4.80 and 4.83 for anthanthrene and benzo[ghi]perylene. Minor components identified in fraction 5 include binaphthyl isomers and methyl substituted 252 isomers (or dibenzofluorenes).

Fraction 6 contains four molecular weight 276 isomers (indeno[1,2,3-cd]-pyrene is the major peak and traces of benzo[ghi]perylene overlap from fraction 5) and four unknown constituents of molecular weight 300. Five isomers of molecular weight 278 are the major components of fraction 7. Three compounds of molecular weight 300 and four dibenzopyrene isomers (MW 302) are the minor components. Additional dibenzopyrene isomers (5 peaks) are the major components of fraction 8.

A comparison of the gas chromatograms obtained from the normal-phase LC fractionation and the chromatogram of the total fraction PAH (see Figure 2) illustrates the advantage of multidimensional LC/GC for detailed characterization of complex PAH mixtures.

FRACTIONATION OF WASHINGTON AIR PARTICULATE MATERIAL FOR CHEMICAL ANALYSIS AND BIOLOGICAL TESTING

After preliminary investigation of the classical acid/base/neutral liquid-liquid fractionation scheme for characterization of the organic constituents on the Washington dust sample, a meeting was held with EPA personnel to discuss the progress and future approach to validation of an overall fractionation scheme. As a result a plan was proposed to more effectively and thoroughly evaluate a fractionation scheme in conjunction with Battelle Columbus Laboratories (BCL) and Research Triangle Institute (RTI). The classical liquid-liquid partition scheme described by Pellizzari *et al.* (26) was modified to include silica gel column chromatography of the neutral fraction to isolate [1] aliphatic hydrocarbons, [2] aromatic compounds, [3] moderately polar compounds, and [4] highly polar compounds, in addition to the initial acidic and basic fractions, for a total of six fractions. The fractionation scheme utilized by BCL to isolate the fractions is illustrated in Figure 18. Individual fractions were analyzed by laboratories with expertise for a particular class of compounds as follows: aromatic fraction (NBS), acidic fraction (RTI), and moderately polar and highly polar fractions (BCL). The basic fraction was qualitatively characterized by both RTI and NBS, and BCL determined nitro-PAH in the aromatic fraction. These fractions were also tested for biological activity by EPA. The aromatic fraction was further fractionated by NBS on an aminosilane column to isolate and characterize the polycyclic aromatic hydrocarbon content. The resulting fractions were also tested for biological activity by EPA.

A 130 mg aromatic fraction (obtained from the extraction of ~30 g of Washington dust) was received from BCL. This sample was fractionated on an aminosilane column to obtain four fractions as shown in Figure 19. Aliquots of these fractions were sent to EPA for biological testing. The results of the biological testing are summarized in Table 9 (27). The majority of the activity was found in fractions 1 and 2. The PAH are contained in fraction 1, however, other components are also present as indicated by the presence of

TABLE 9. RESULTS OF BIOLOGICAL TESTING ON FRACTIONS FROM WASHINGTON AIR PARTICULATE MATERIAL (27)

Fraction	Mass	Activity (revertants/μg) -S-9 activation	Activity (revertants/μg) +S-9 activation	Weighted Mutagenicity -S-9	Weighted Mutagenicity +S-9	% Mutagenicity -S-9	% Mutagenicity +S-9
NBS Normal-Phase LC							
0	32%	N.T.[a]	N.T.	N.T.	N.T.	N.T.	N.T.
1	24%	5.1	11.9	1.2	2.9	56.8	45
2	40%	2.3	8.7	0.9	3.5	42.7	54.8%
3	4%	0.29	0.35	0.01	0.01	0.5	0.2
Total	100%			2.11	6.41		
BCL Fractionation							
Hex/Benz (1)		2.06	2.86				
Hex/Benz (2)		1.96	2.95				
Hex/Benz (NBS)		0.22	1.42				
Hex/Benz (1) (stored)		0.24	3.50				
Hex/Benz (2) (stored)		0.16	1.66				

[a] N.T. = not tested, assumed to have no activity.

biological activity without S-9 activation (PAH require activation for mutagenicity). The total mutagenic activity without activation of all the fractions compares favorably with the activity of two hexane/benzene fractions prior to fractionation on the aminosilane column (2.11 vs. 2.06 and 1.96). However, the activity of the hex/benz fractions tested after ∿60 days was found to be significantly lower than the initial testing.

The results of the qualitative chemical characterization indicated that the PAH were contained in fraction 1 as expected. The most characteristic features of the mass chromatogram was the broad unresolved hump with a number of superimposed peaks. The spectra for over 100 of these peaks were examined; however many of the spectra did not provide useful information because of the complex superimposed background spectra. The majority of the peaks in the chromatogram gave characteristic PAH spectra. These compounds are not reported here since the PAH constituents were characterized extensively in earlier analyses (see Table 8). The additional peaks (several present in similar quantities as the PAH) gave mass spectra characteristic of methyl esters of fatty acids and PAH quinones/ketones, (e.g., benzaldehyde, fluorenone, methylmyristate, methylpalmitate methylstearate or oleate, anthraquinone, and benzanthrone). In addition, other experiments involving the isolation of the PAH fraction from the Washington dust indicate that the amount of PAH material should be only about 25 percent of the mass collected in fraction 1. Modifications to the fractionation scheme such as a cyclohexane/DMF/H_2O partition of the aromatic fraction prior to normal-phase LC separation on the amine column may provide a "pure" PAH fraction. GC-MS analysis of fraction 2 revealed the presence of numerous phthalates, methyl silicone artifacts (presumably from the aminosilane column), aza-PAH (molecular weight 227) carbazole, and two benzanthrone isomers, superimposed on an unresolved hump. The largest peak was dioctylphthalate. Analysis of fraction 3 indicated that the only constituents present were probably due to artifacts from the LC column. The basic fraction was analyzed by GC with identifications by MS. The gas chromatogram is shown in Figure 20 and the tentative identifications are listed in Table 10.

CONCLUSIONS

Biological testing can provide valuable information concerning the potential health hazards associated with various fractions isolated from air particulate material. Since the polycyclic aromatic hydrocarbon (PAH) fraction has been found to possess mutagenic activity, it is important to develop methods for complete characterization of the fraction. The analytical methods described in this work illustrate the capabilities of liquid and gas chromatographic techniques for the separation, identification, and quantitation of individual components in this complex PAH mixture. Future work will emphasize the chemical characterization and biological testing of various PAH fractions to identify which PAH fractions (or compounds) are responsible for the mutagenic activity in air particulate samples.

TABLE 10. AZA-ARENES IDENTIFIED IN BASIC FRACTION OF WASHINGTON URBAN DUST

Peak #	Mol. Wt.	Compound
1	121	C_3-substituted pyridine
2	129	isoquinoline
3	143	C_1-substituted quinoline/isoquinoline
4	143	C_1-substituted quinoline/isoquinoline
5	143	C_1-substituted quinoline/isoquinoline
6	143	C_1-substituted quinoline/isoquinoline
7	157	C_2-substituted quinoline/isoquinoline
8	157	C_2-substituted quinoline/isoquinoline
9	179	Benzoquinoline
10	179	Benzoquinoline
11	179	Benzoquinoline
12	194	Caffeine
13	---	Unknown
14	---	Unknown

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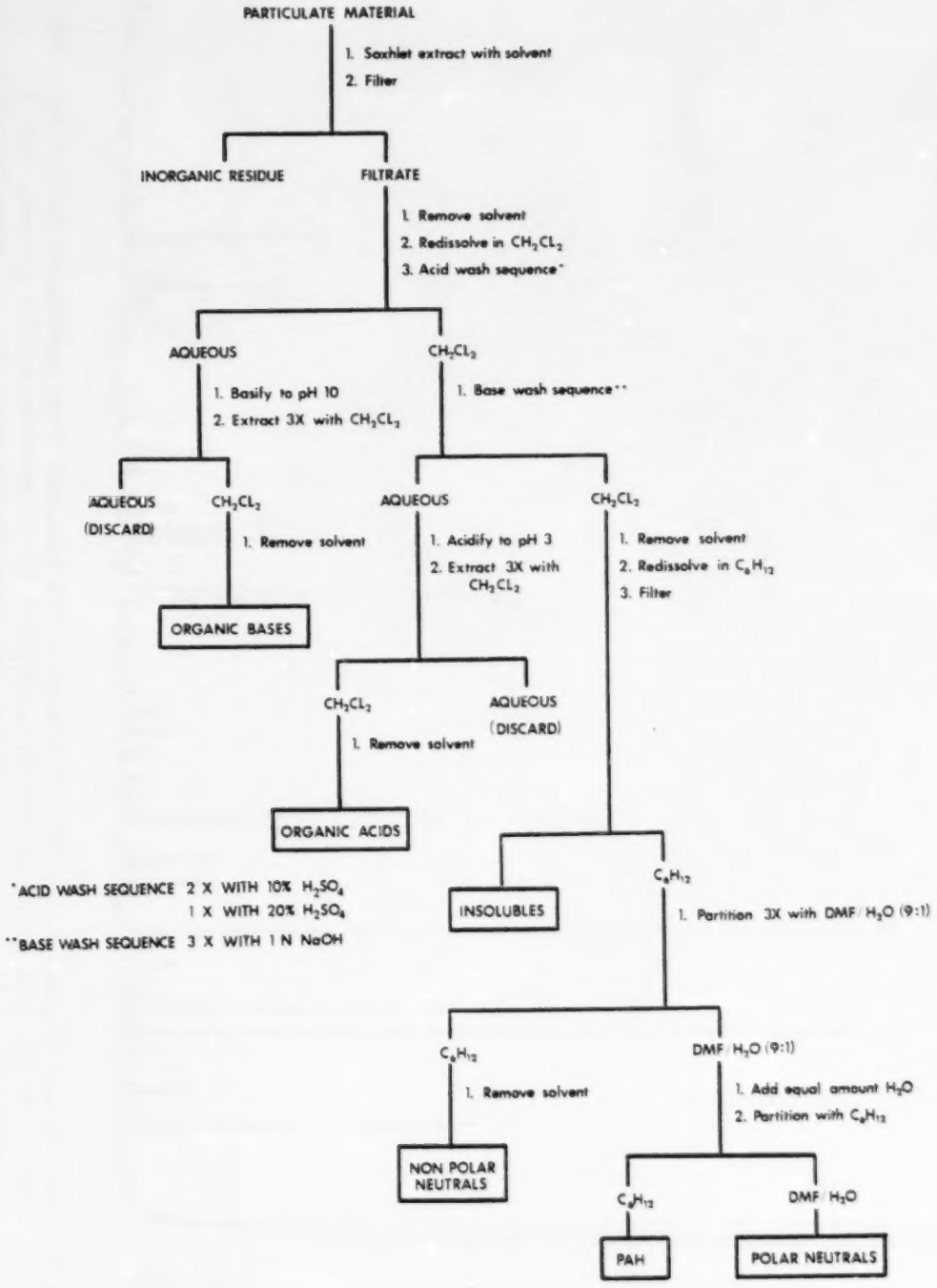

Figure 1. General scheme for extraction and fractionation of air particulate material for chemical and biological characterization (adapted from reference 26).

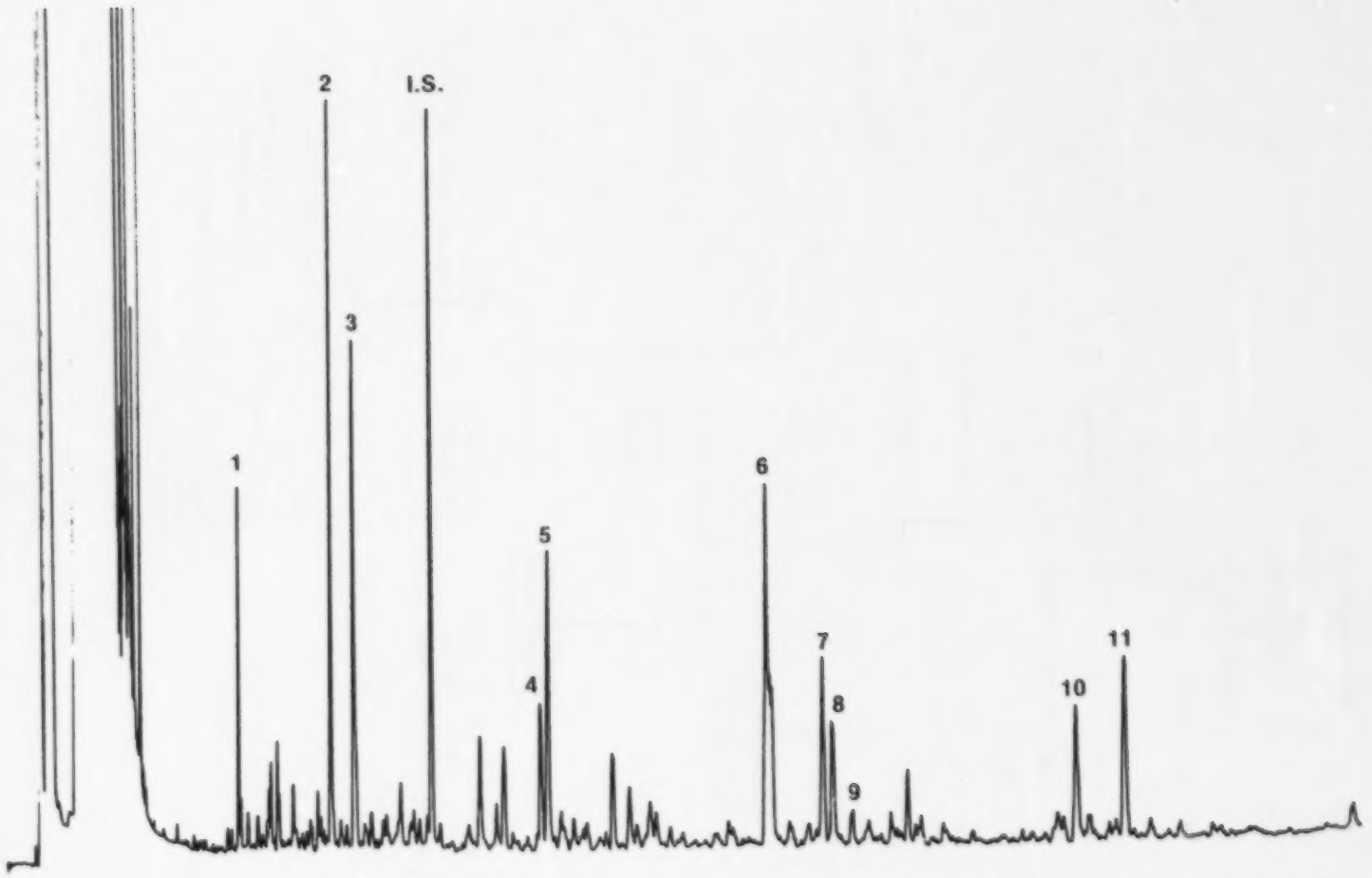

Figure 2. Gas chromatographic separation of PAH fraction isolated from Washington urban dust (SRM 1649). Peaks identified as (1) phenanthrene, (2) fluoranthene, (3) pyrene, (I.S.) 1-methylpyrene, (4) benz[a]anthracene, (5) chrysene/triphenylene, (6) benzofluoranthenes, (7) benzo[e]pyrene, (8) benzo[a]pyrene, (9) perylene, (10) indeno[1,2,3-cd]pyrene, and (11) benzo[ghi]perylene. Conditions: column – 30 m x 0.25 mm i.d. SE-52 fused silica capillary; temperature program – 200 °C (3 min hold) programmed to 300 °C at 2 °C/min.

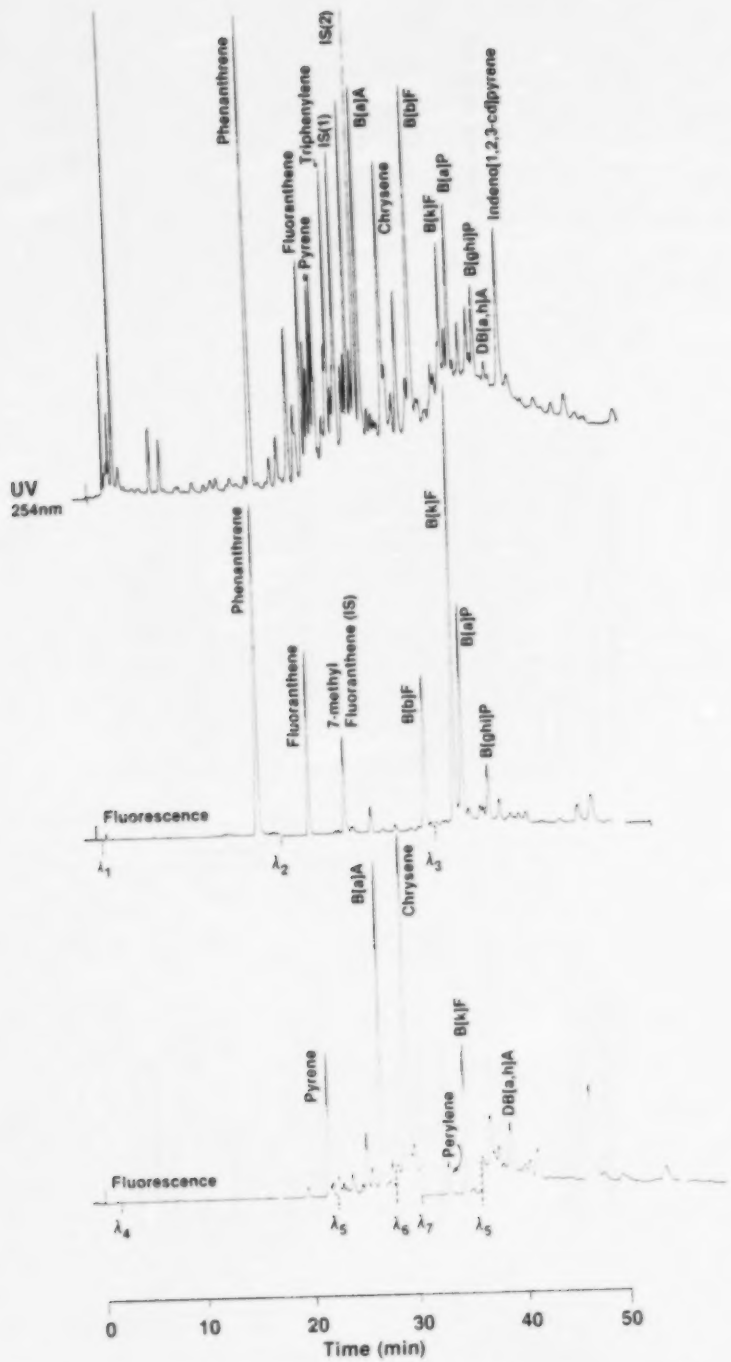

Figure 3. Reversed-phase liquid chromatograms of total PAH fraction from Washington urban dust (SRM 1649). Sample cleanup included normal-phase LC. Upper chromatogram: UV detection at 254 nm; middle and lower chromatograms: fluorescence detection at conditions described in Table 2. (See Experimental Section for conditions.)

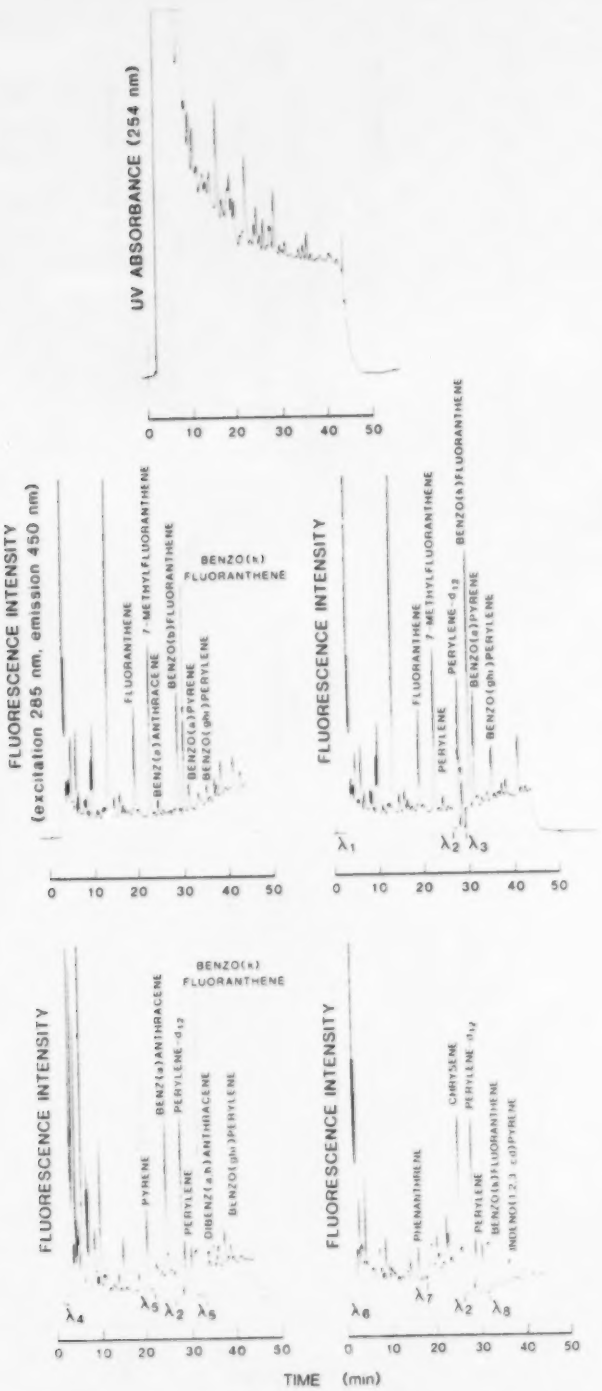

Figure 4. Reversed-phase LC analysis of PAH from Washington urban dust (SRM 1649). No normal-phase LC cleanup. Fluorescence detection at conditions described in Table 3. (See Experimental Section for conditions.)

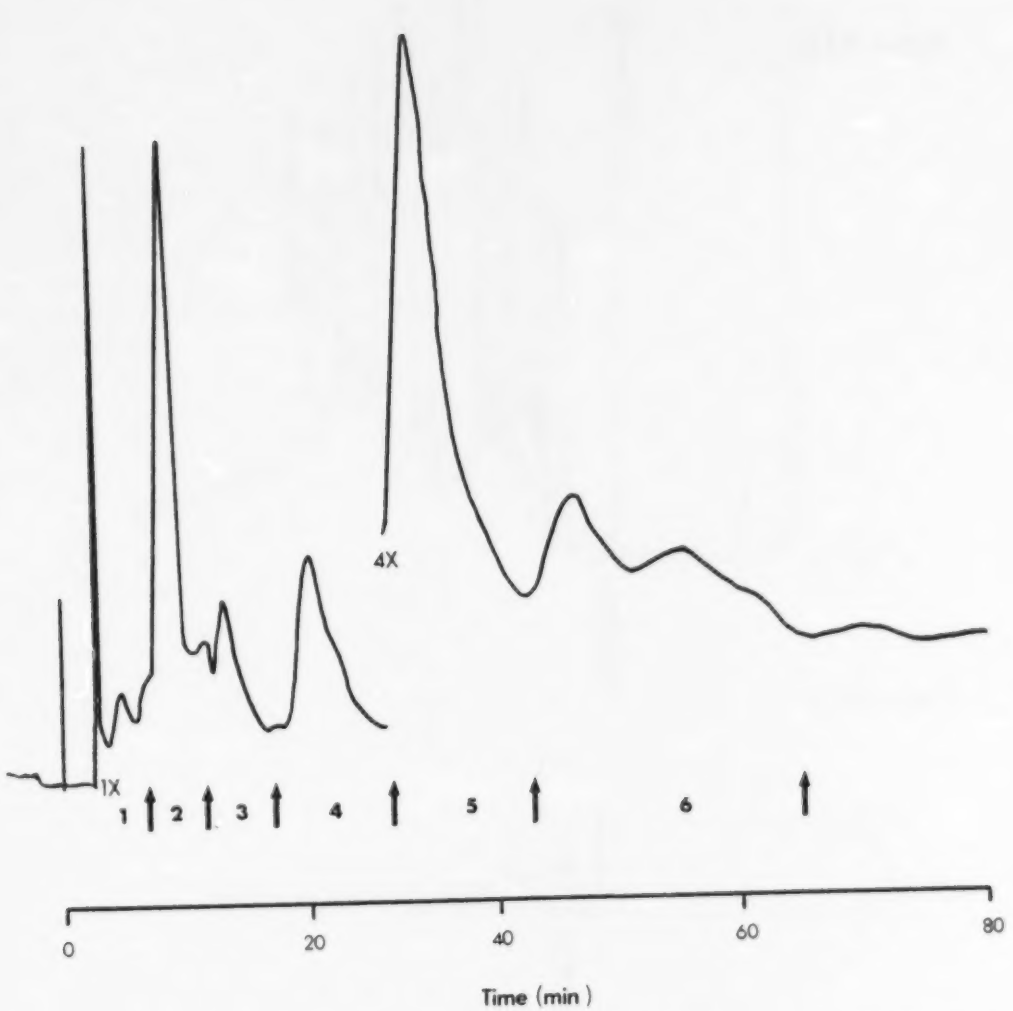

Figure 5. Normal-phase LC separation of PAH isolated from Washington urban dust for reversed-phase LC analysis. Conditions: semi-prep μBondapak NH_2 column; mobile phase — 1% methylene chloride in hexane at 5 mL/min; UV detection at 254 nm.

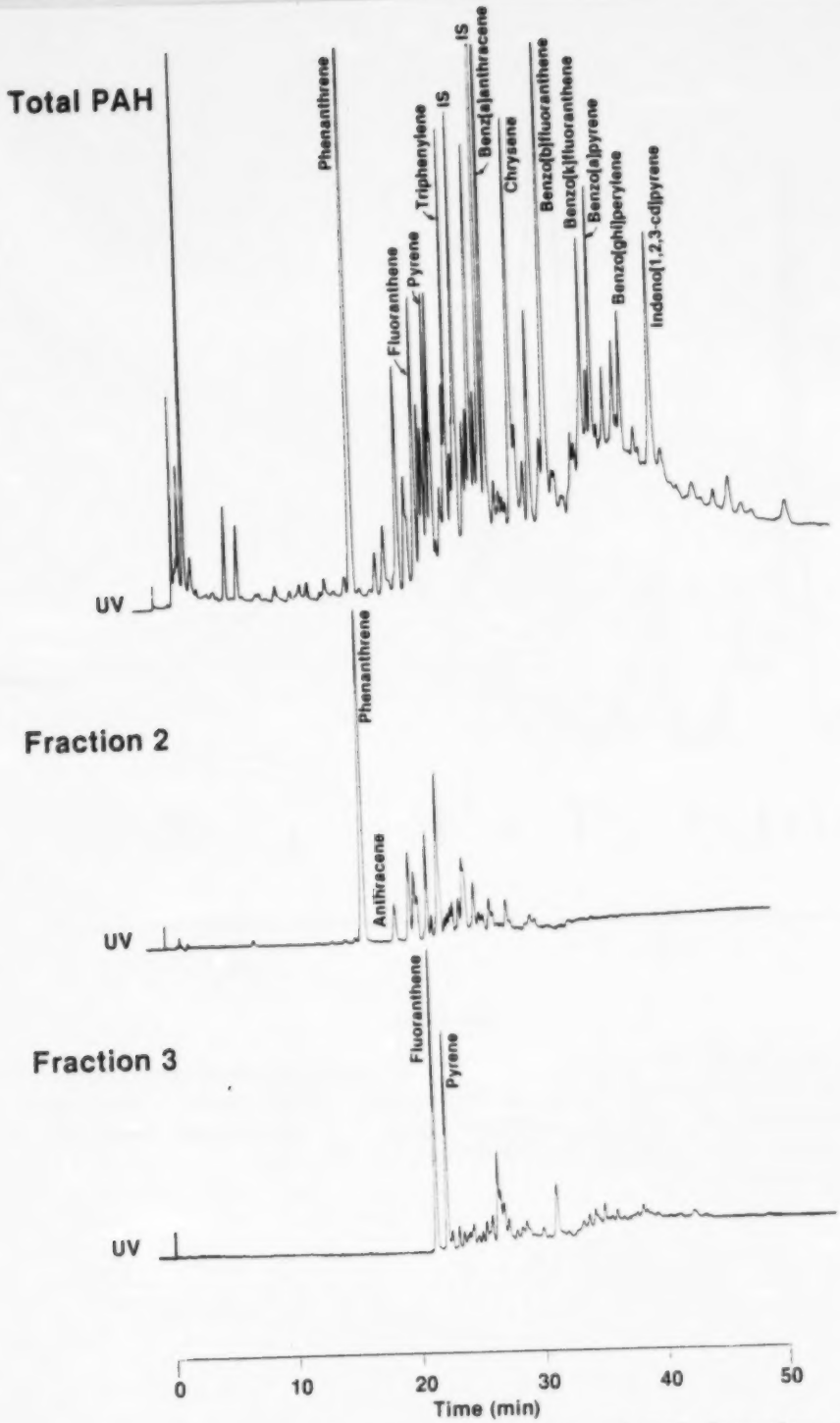

Figure 6. Reversed-phase LC separations of total PAH fraction and fractions 2 and 3 obtained from normal-phase LC fractionation of PAH from Washington urban dust (see Figure 5). Conditions: column — Vydac 201TP 5μ; mobile phase — linear gradient from 40-100% acetonitrile in water at 1%/min at 1 mL/min; UV detection at 254 nm.

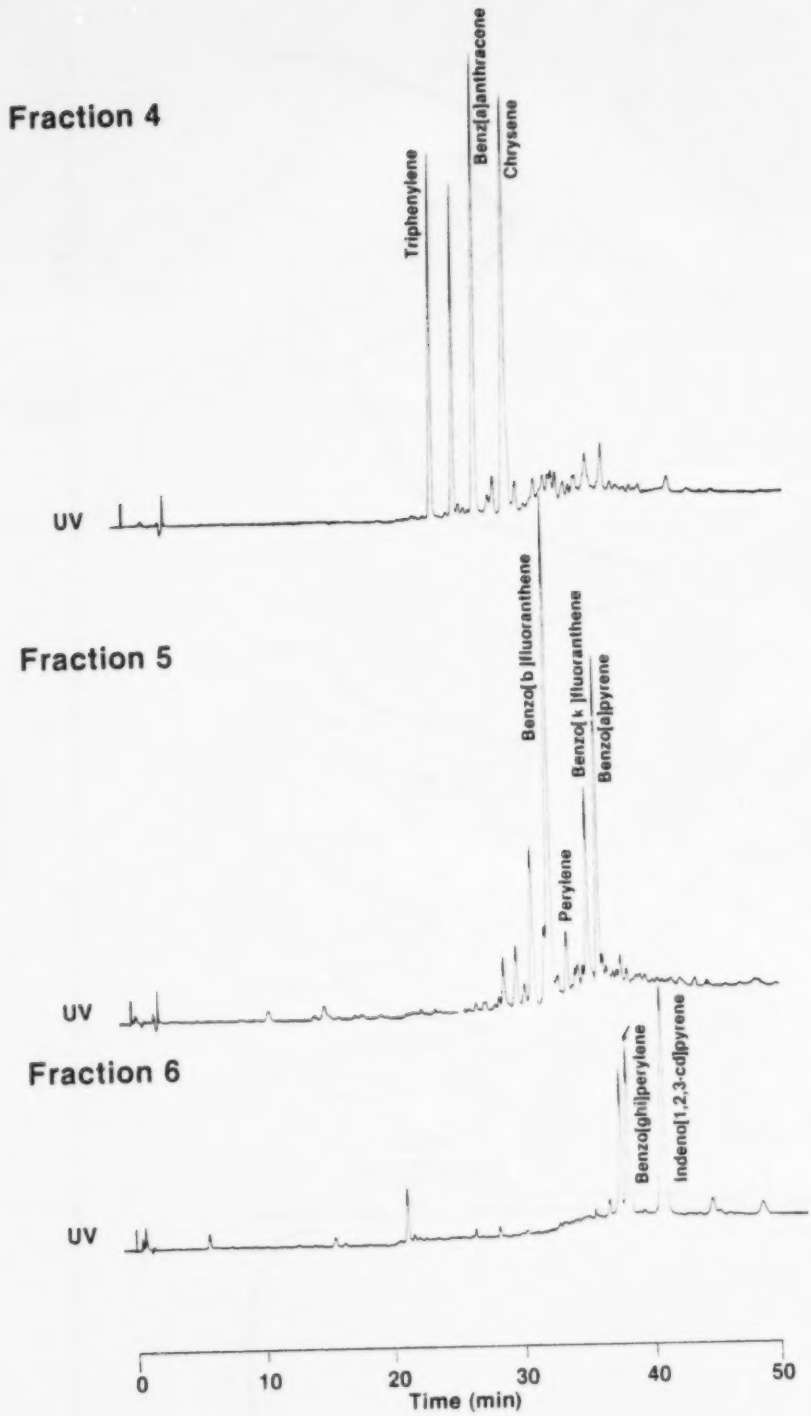

Figure 7. Reversed-phase LC separation of fractions 4-6 obtained from normal-phase LC fractionation of PAH from Washington urban dust. Conditions: same as Figure 6.

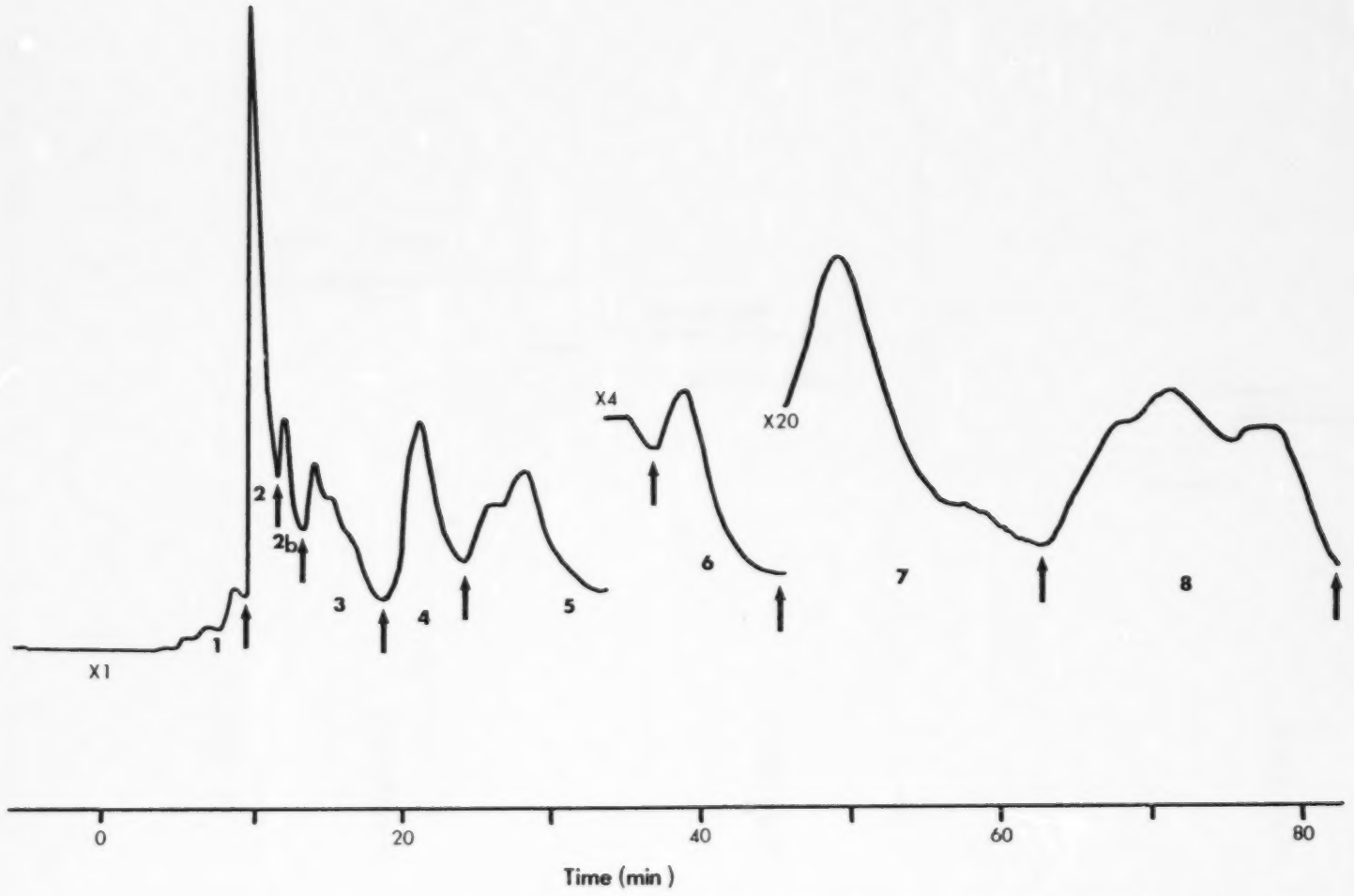

Figure 8. Normal-phase LC separation of PAH isolated from Washington urban dust for GC-MS analysis. Analysis conditions: semi-prep μBondapak NH_2, mobile phase — 0.5% methylene chloride in pentane at 4 mL/min; UV detection at 254 nm.

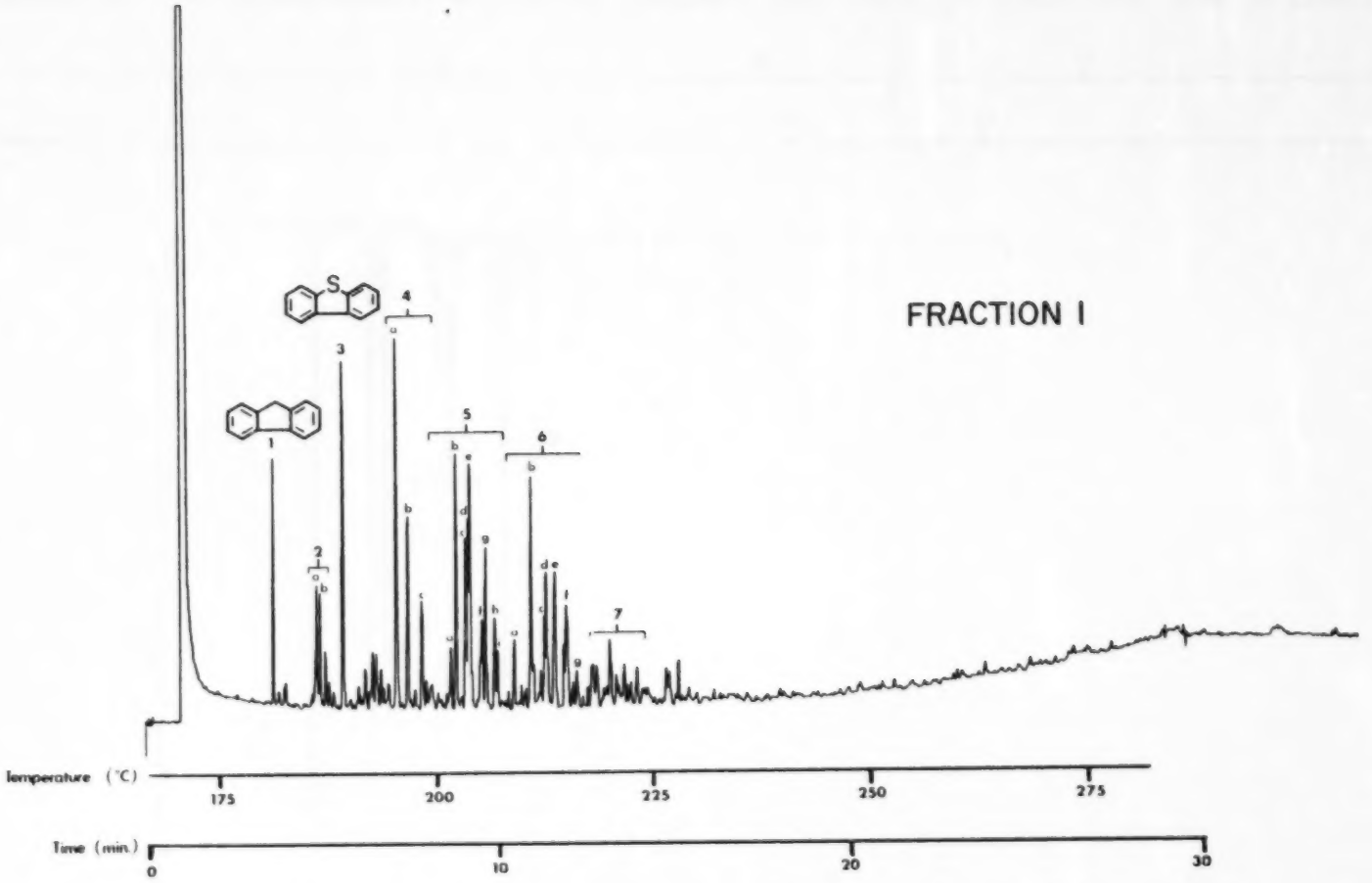

Figure 9. Gas chromatographic analysis of fraction 1 from Figure 8. Conditions: column — 30m x 0.25mm i.d. SE-52 fused silica capillary; temperature program — 175 °C (2 min hold) programmed to 280 °C at 4 °C/min.

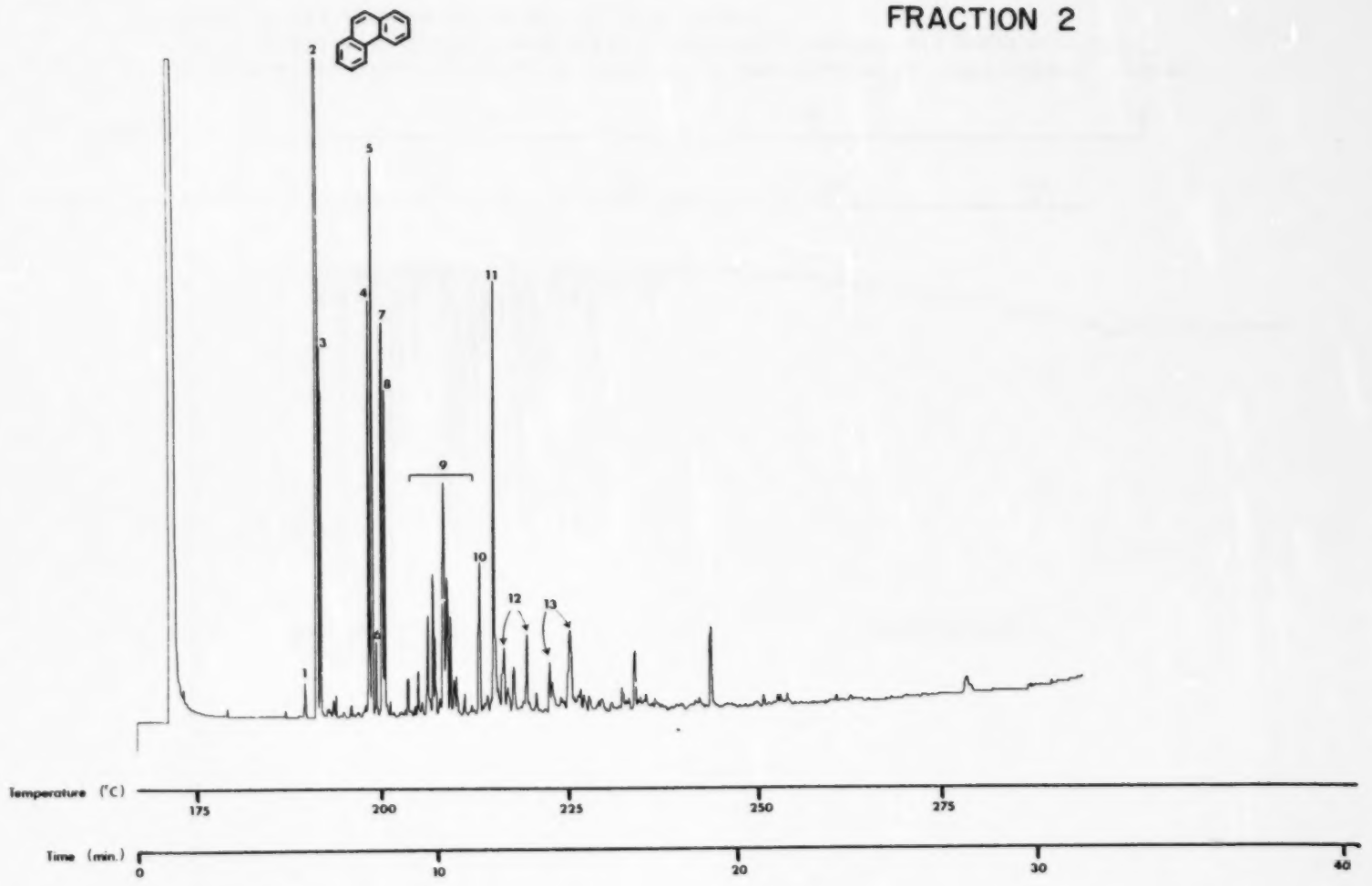

Figure 10. Gas chromatographic analysis of fraction 2 from Figure 8. Conditions: same as Figure 9.

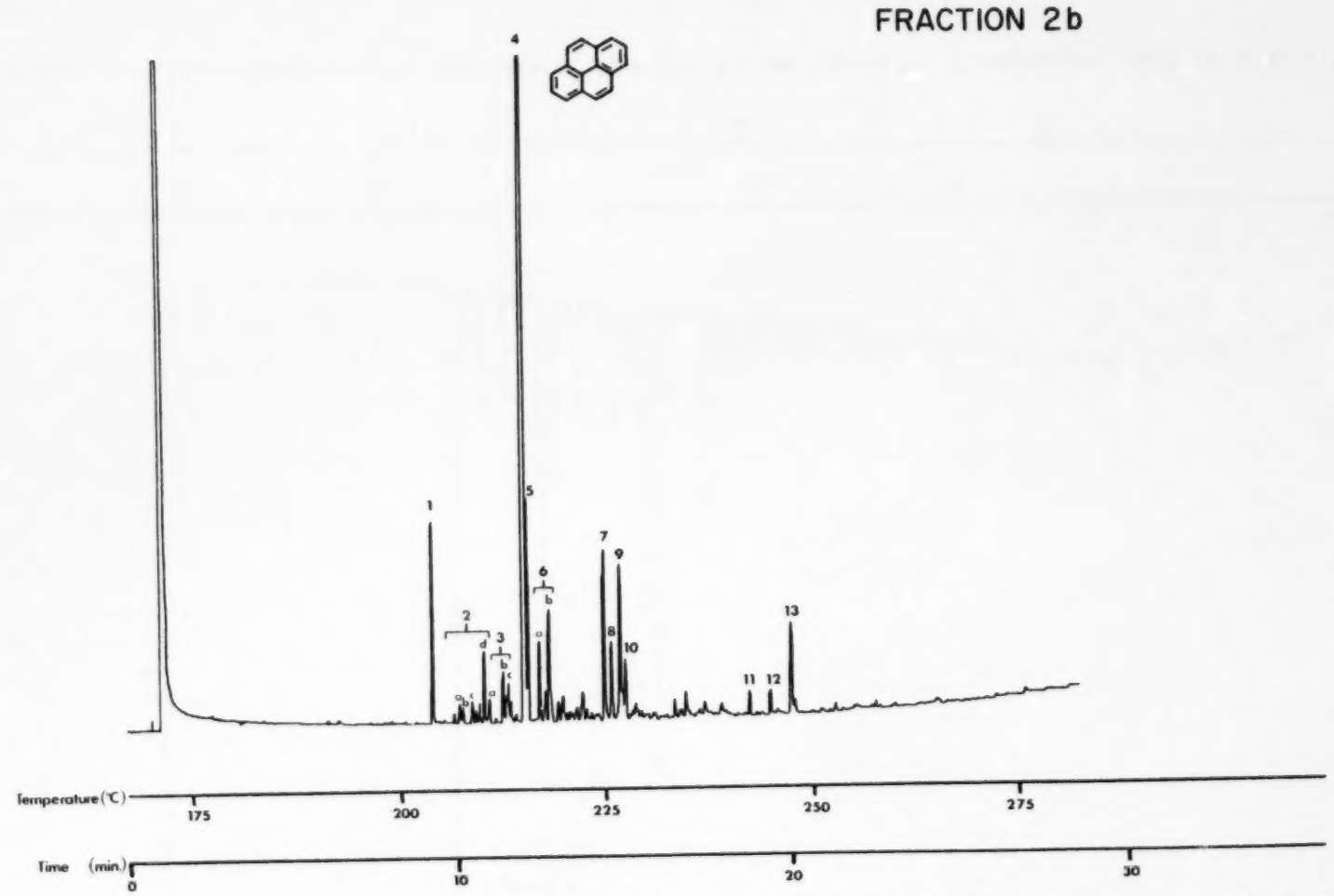

Figure 11. Gas chromatographic analysis of fraction 2b from Figure 8. Conditions: same as Figure 9.

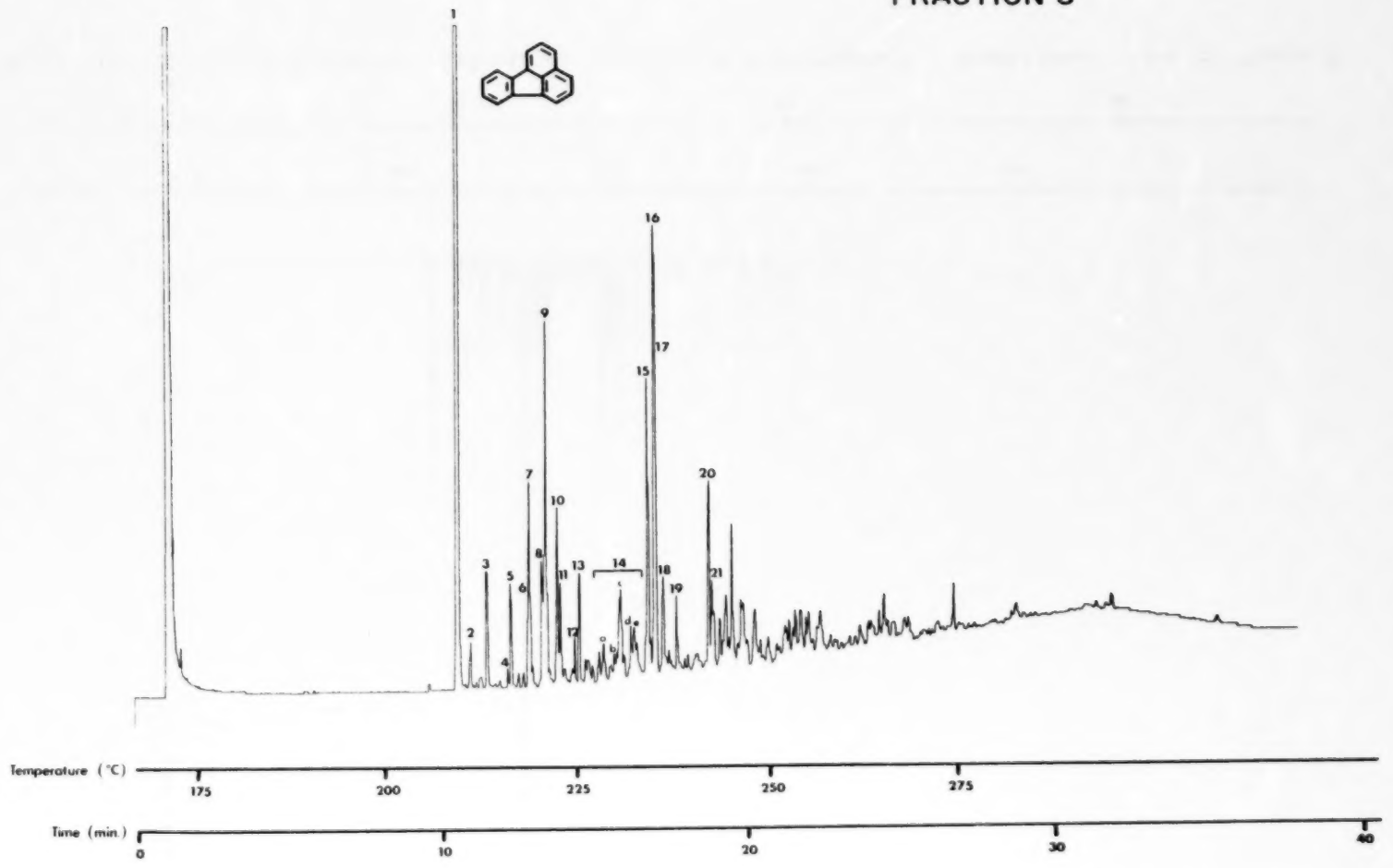

Figure 12. Gas chromatographic analysis of fraction 3 from Figure 8. Conditions: same as Figure 9.

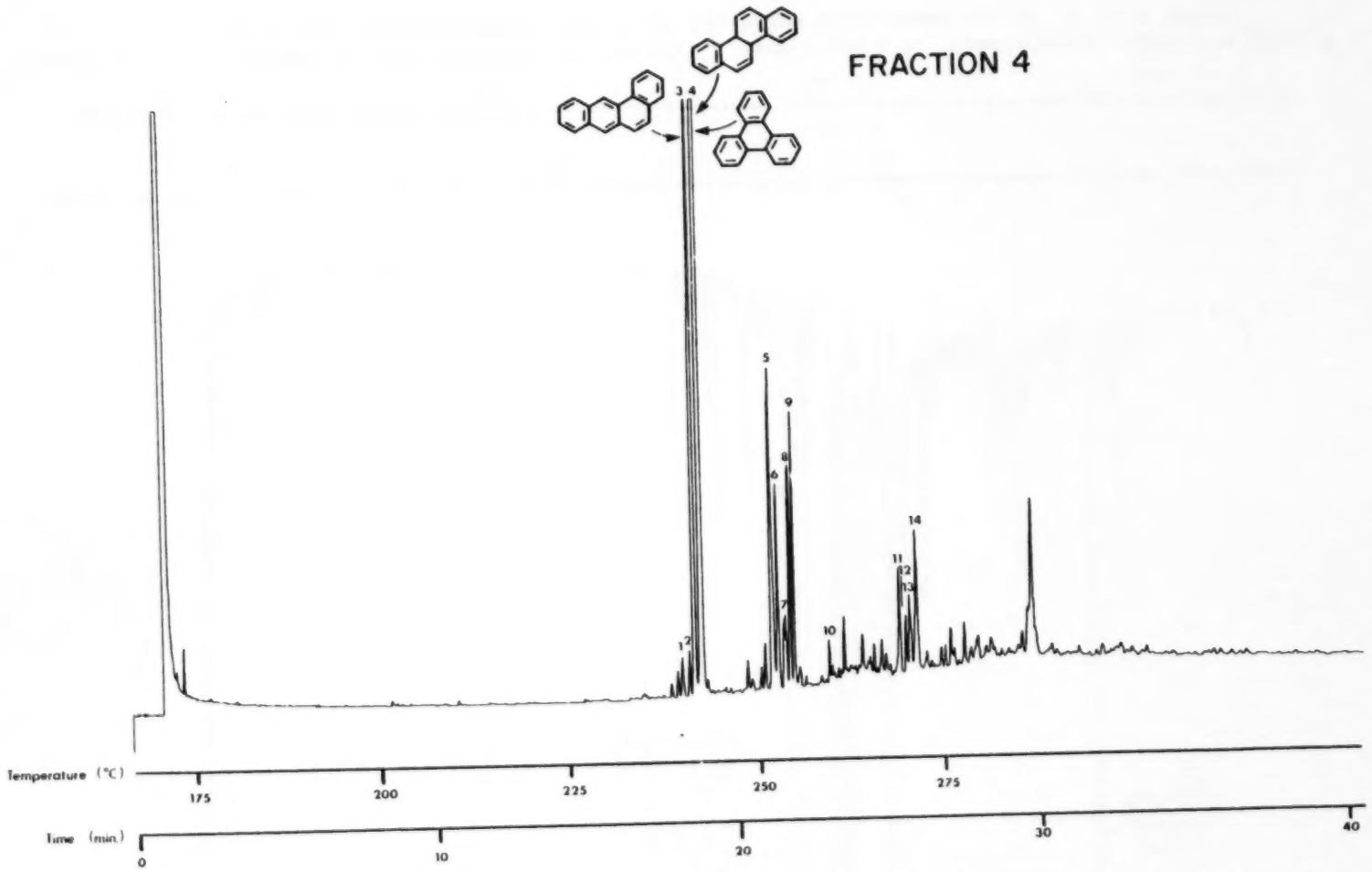

Figure 13. Gas chromatographic analysis of fraction 4 from Figure 8. Conditions: same as Figure 9.

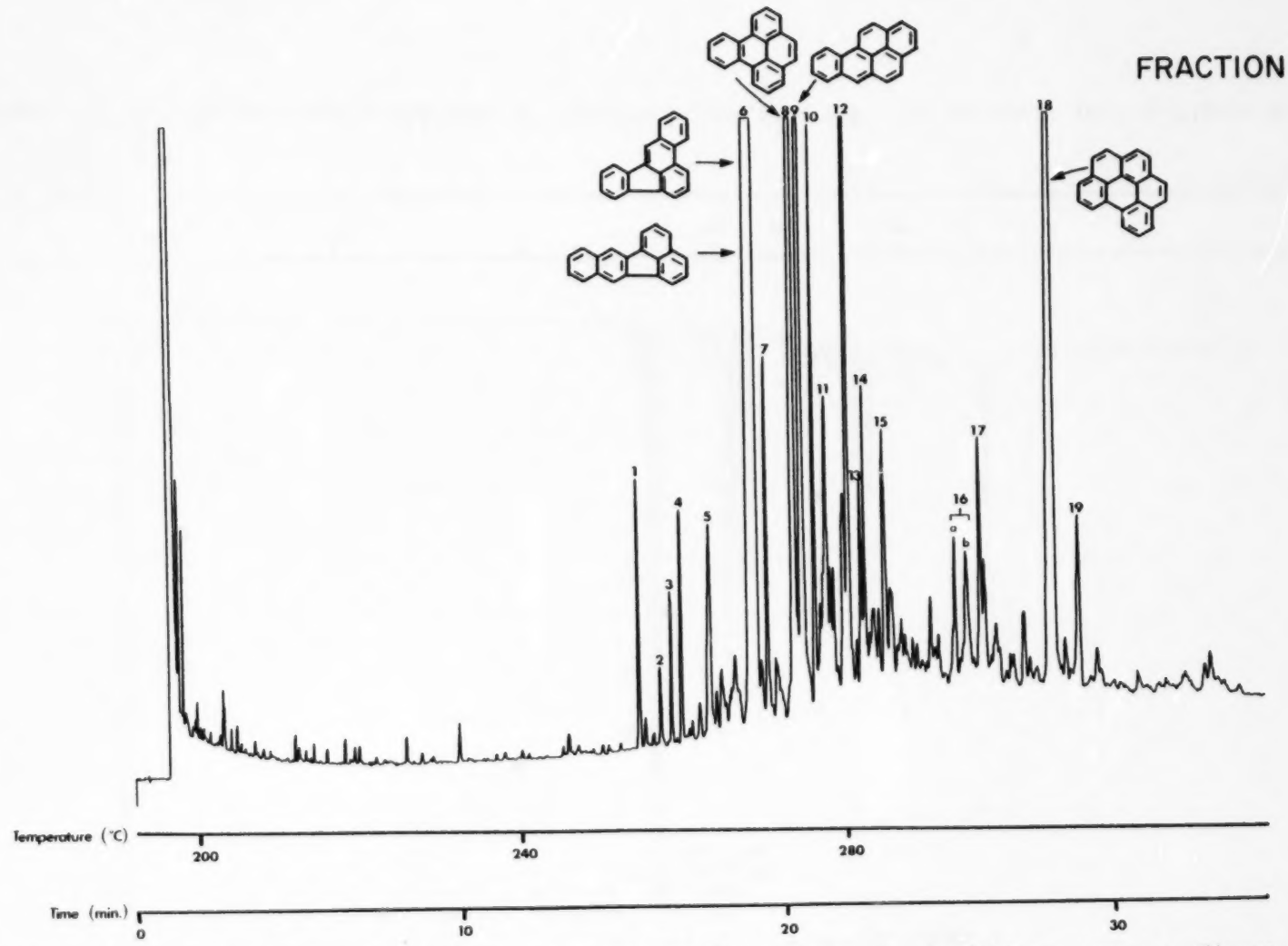

Figure 14. Gas chromatographic analysis of fraction 5 from Figure 8. Conditions: same as Figure 9 except temperature program: 200 °C (2 min hold) programmed to 280 °C at 4 °C/min.

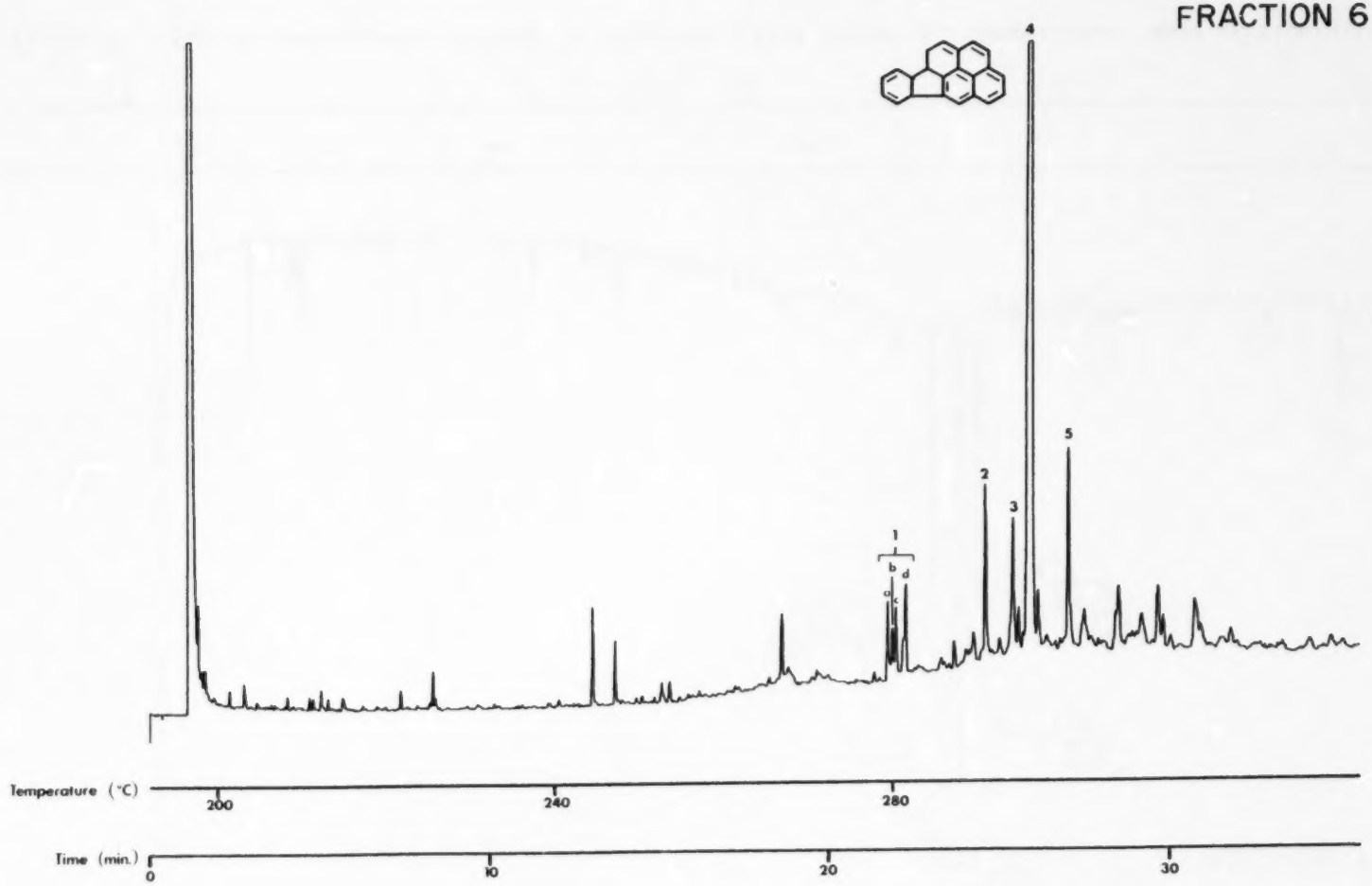

Figure 15. Gas chromatographic analysis of fraction 6 from Figure 8. Conditions: same as Figure 14.

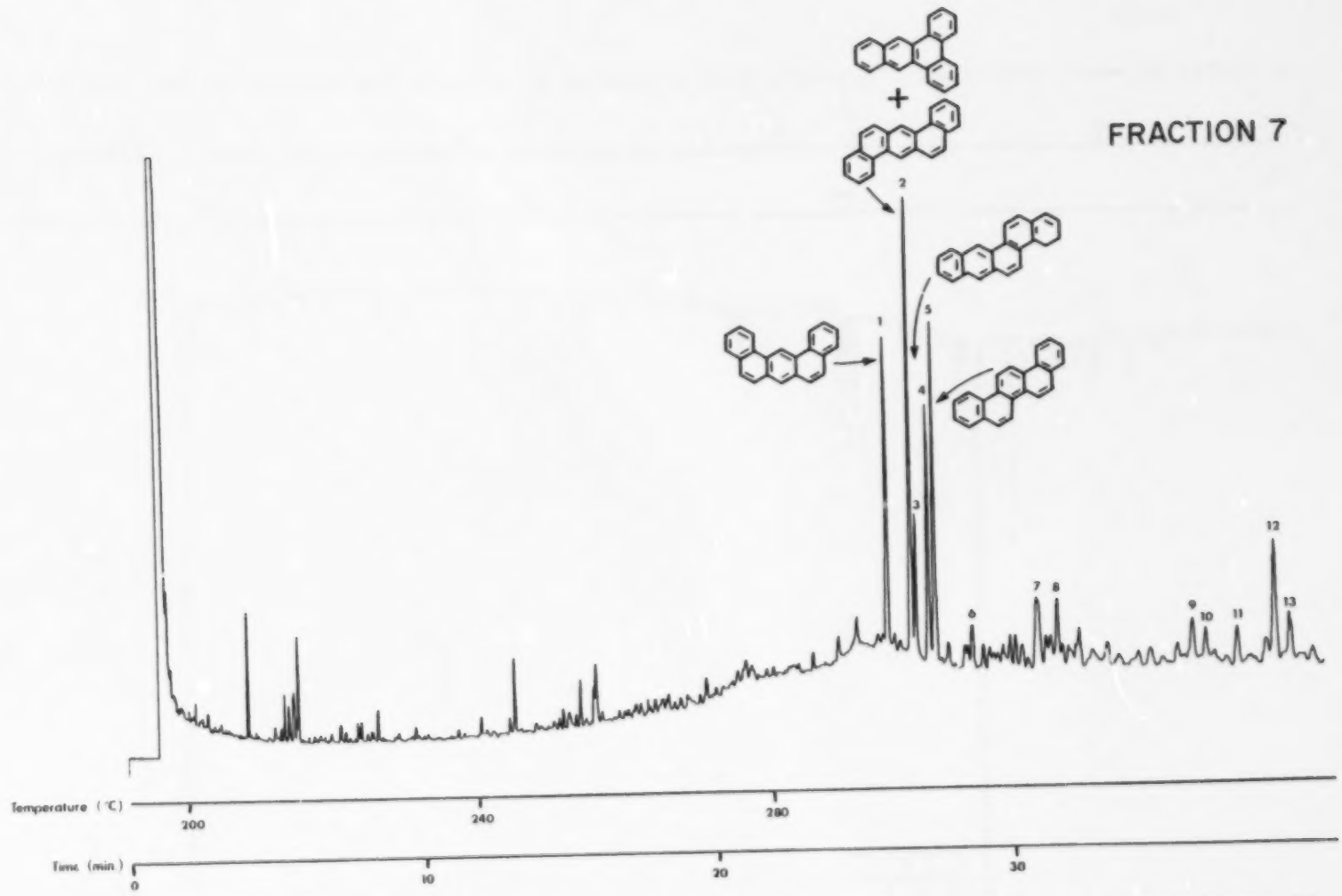

Figure 16. Gas chromatographic analysis of fraction 7 from Figure 8. Conditions: same as Figure 14.

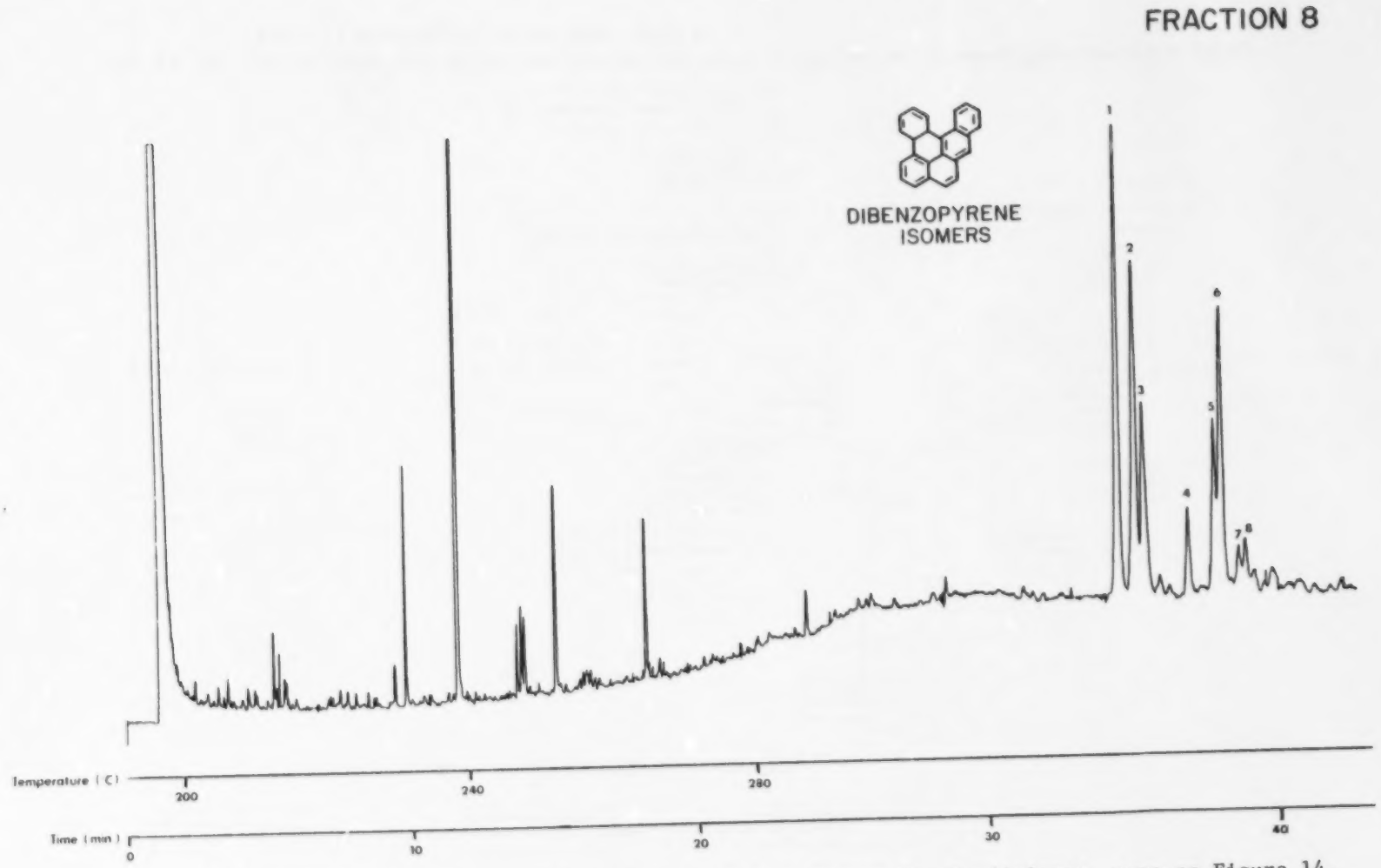

Figure 17. Gas chromatographic analysis of fraction 8 from Figure 8. Conditions: same as Figure 14.

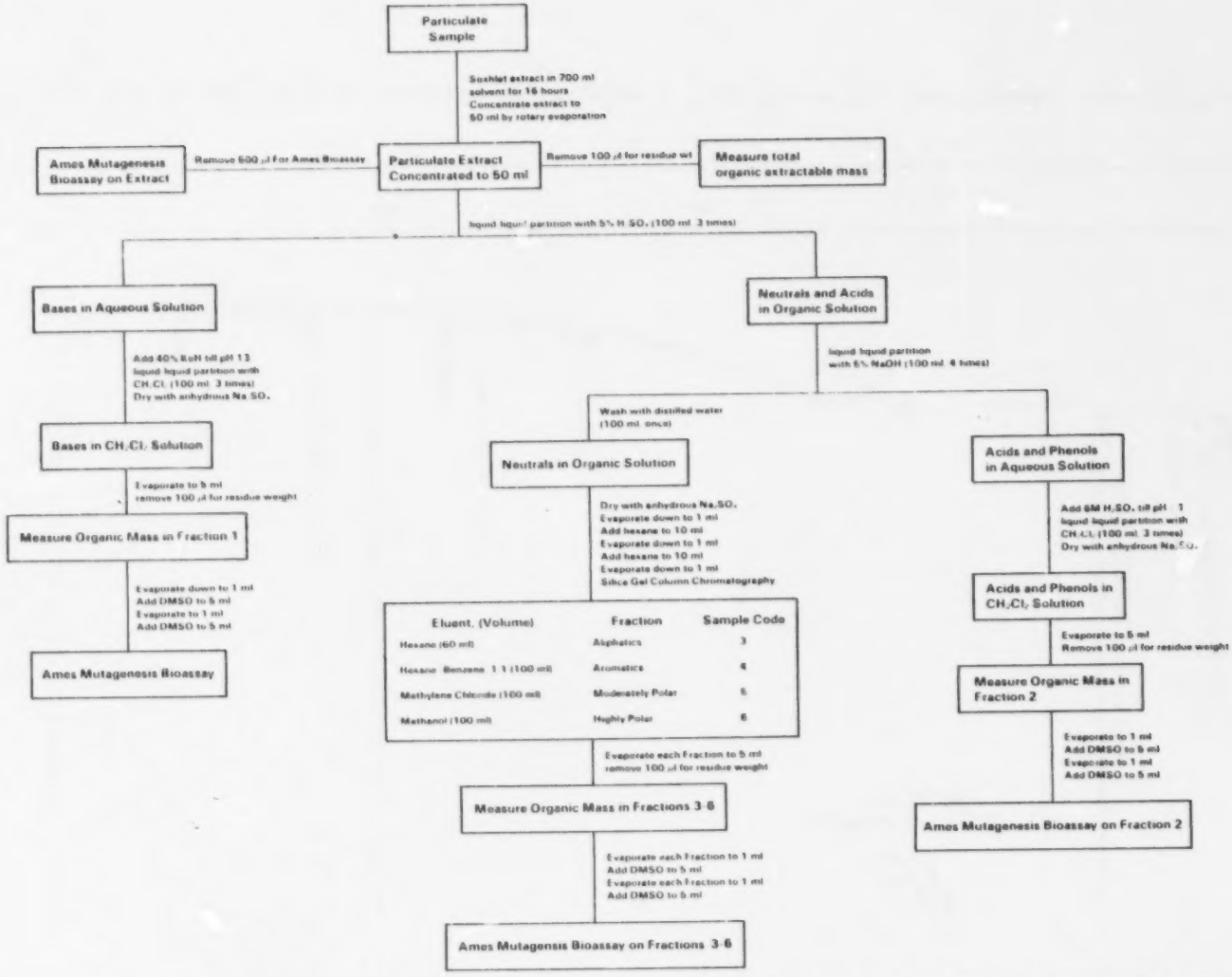
Particulate Sample
Soxhlet extract in 700 ml solvent for 16 hours
Concentrate extract to 50 ml by rotary evaporation
Ames Mutagenesis Bioassay on Extract
Remove 600 µl For Ames Bioassay
Particulate Extract Concentrated to 50 ml
Remove 100 µl for residue wt
Measure total organic extractable mass
liquid liquid partition with 5% H2SO4 (100 ml 3 times)
Bases in Aqueous Solution
Neutrals and Acids in Organic Solution
Add 40% KoH till pH 13
liquid liquid partition with CH2Cl2 (100 ml 3 times)
Dry with anhydrous Na2SO4
liquid liquid partition with 5% NaOH (100 ml 4 times)
Bases in CH2Cl2 Solution
Wash with distilled water (100 ml once)
Neutrals in Organic Solution
Acids and Phenols in Aqueous Solution
Evaporate to 5 ml
remove 100 µl for residue weight
Measure Organic Mass in Fraction 1
Dry with anhydrous Na2SO4
Evaporate down to 1 ml
Add hexane to 10 ml
Evaporate down to 1 ml
Add hexane to 10 ml
Evaporate down to 1 ml
Silica Gel Column Chromatography
Add 6M H2SO4 till pH 1
liquid liquid partition with CH2Cl2 (100 ml 3 times)
Dry with anhydrous Na2SO4
Acids and Phenols in CH2Cl2 Solution
Evaporate down to 1 ml
Add DMSO to 5 ml
Evaporate to 1 ml
Add DMSO to 5 ml
Ames Mutagenesis Bioassay
Eluent (Volume) | Fraction | Sample Code
Hexane (60 ml) | Aliphatics | 3
Hexane Benzene 1 1 (100 ml) | Aromatics | 4
Methylene Chloride (100 ml) | Moderately Polar | 5
Methanol (100 ml) | Highly Polar | 6
Evaporate each Fraction to 5 ml
remove 100 µl for residue weight
Measure Organic Mass in Fractions 3-6
Evaporate each Fraction to 1 ml
Add DMSO to 5 ml
Evaporate each Fraction to 1 ml
Add DMSO to 5 ml
Ames Mutagenesis Bioassay on Fractions 3-6
Evaporate to 5 ml
Remove 100 µl for residue weight
Measure Organic Mass in Fraction 2
Evaporate to 1 ml
Add DMSO to 5 ml
Evaporate to 1 ml
Add DMSO to 5 ml
Ames Mutagenesis Bioassay on Fraction 2

Figure 18. Extraction and fractionation scheme used by Battelle Columbus Laboratories (BCL) for the Washington urban dust sample.

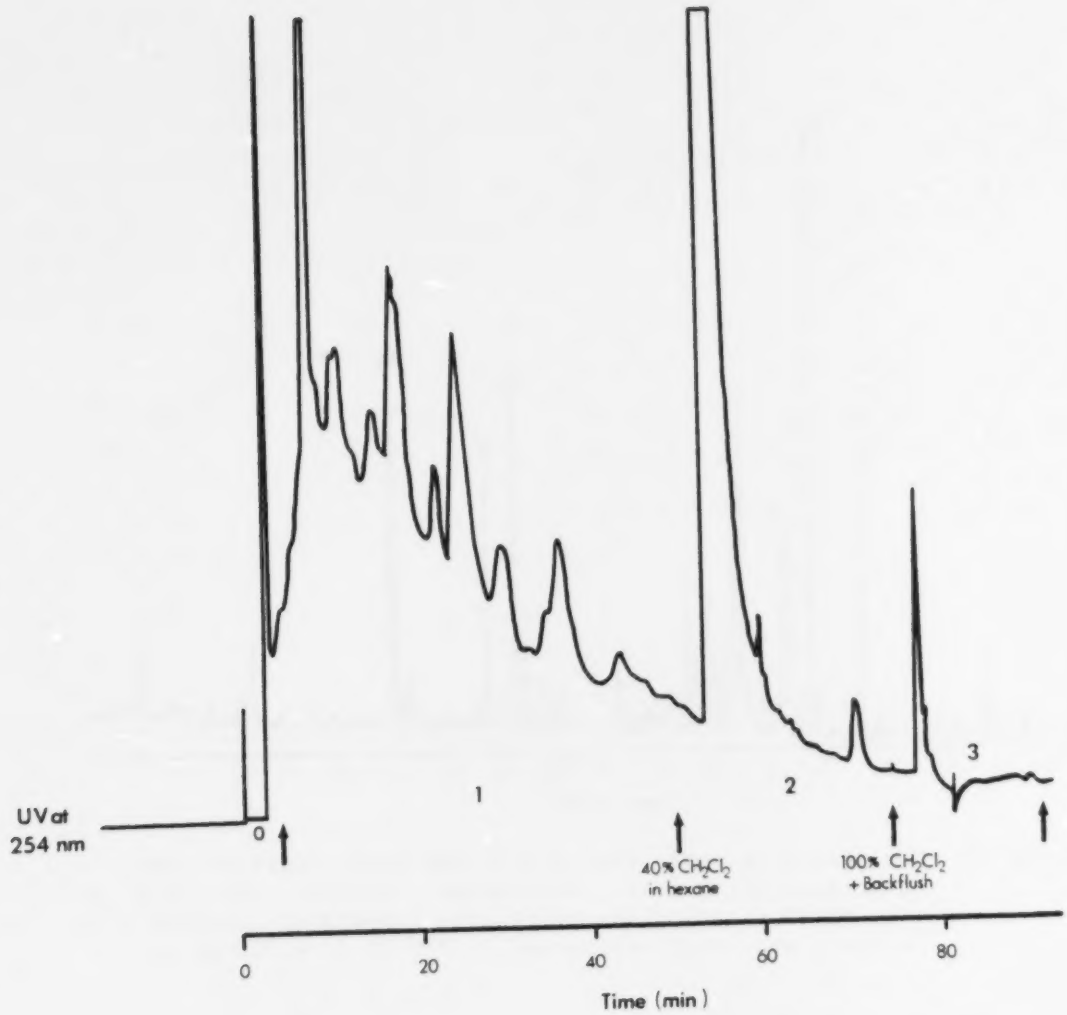

Figure 19. Normal-phase LC separation of the hexane/benzene (aromatic) fraction isolated from Washington urban dust using BCL procedure. Conditions: semi-prep μBondapak NH_2 column, mobile phase — 1% methylene chloride at 5 mL/min, UV detection at 254 nm.

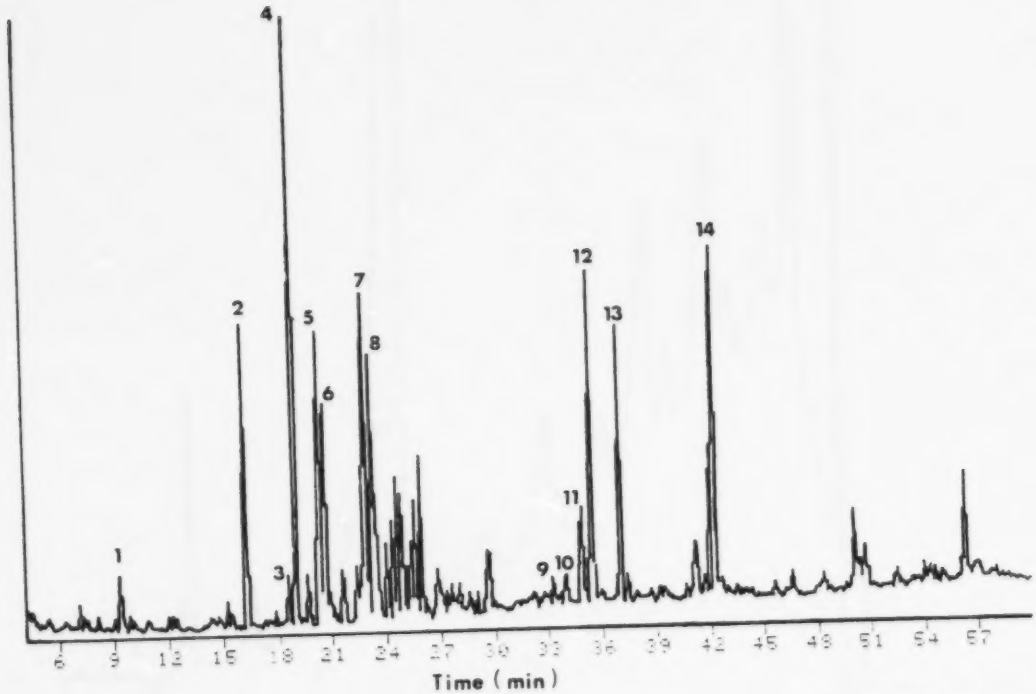

Figure 20. Gas chromatographic analysis of the basic fraction from Washington urban dust. Conditions: column — 30m x 0.25 mm i.d. SE-30 fused silica capillary; temperature program — 50 °C (1 min hold) programmed to 275 °C at 4 °C/min.